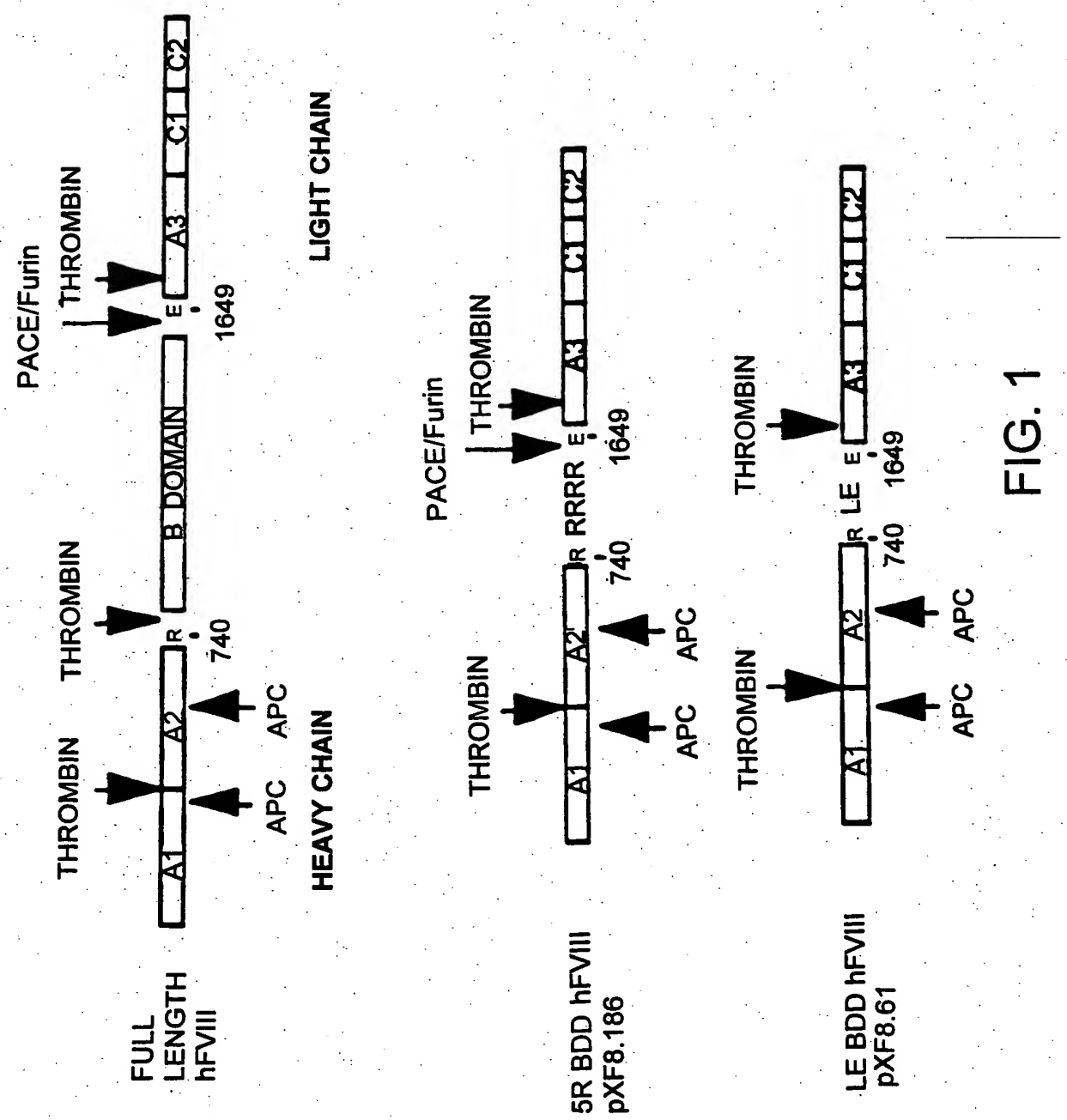
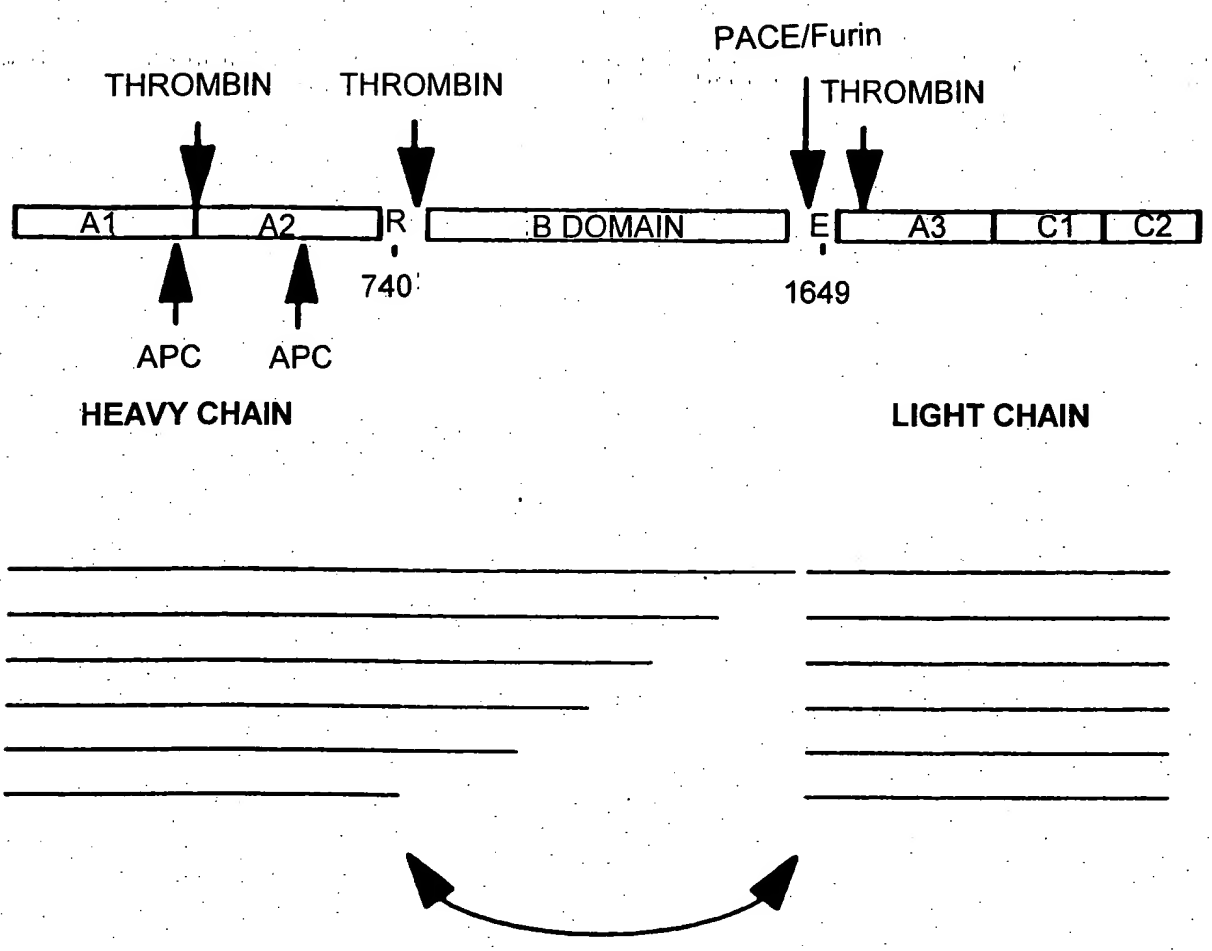


FIG. 1



HETEROGENEITY OF hFVIII IS DUE TO PROTEOLYSIS WITHIN THE B-DOMAIN

FIG. 2

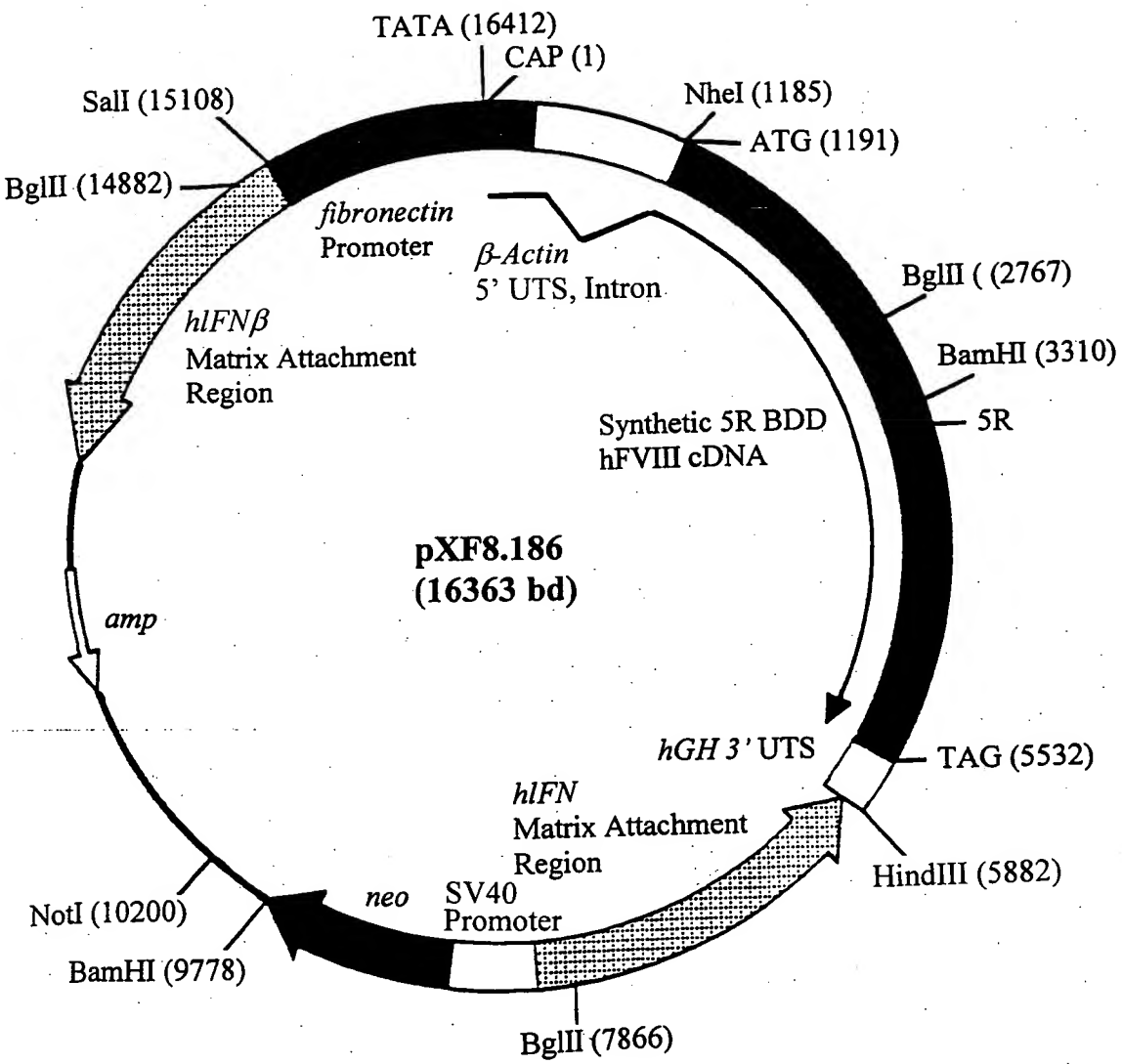

TATA (16412)
CAP (1)
NheI (1185)
ATG (1191)
SalI (15108)
BglII (14882)
fibronectin
Promoter
β-Actin
5' UTS, Intron
BglII ((2767)
BamHI (3310)
5R
hIFNβ
Matrix Attachment
Region
Synthetic 5R BDD
hFVIII cDNA
pXF8.186
(16363 bd)
amp
hGH 3' UTS
TAG (5532)
hIFN
Matrix Attachment
Region
HindIII (5882)
NotI (10200)
neo
SV40
Promoter
BamHI (9778)
BglII (7866)

FIG. 3

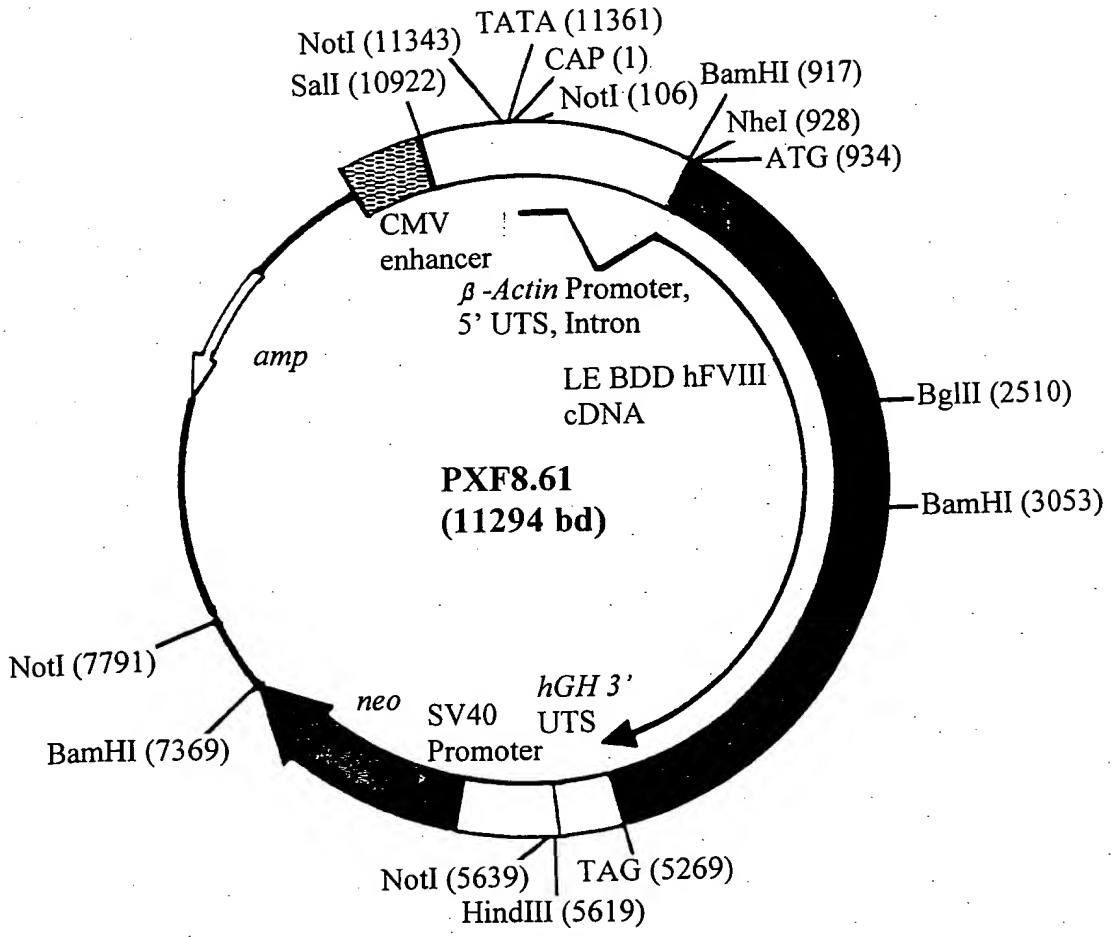

NotI (11343)
TATA (11361)
SalI (10922)
CAP (1)
NotI (106)
BamHI (917)
NheI (928)
ATG (934)
CMV
enhancer
β -Actin Promoter,
5' UTS, Intron
amp
LE BDD hFVIII
cDNA
BglII (2510)
PXF8.61
(11294 bd)
BamHI (3053)
NotI (7791)
neo
SV40
Promoter
hGH 3'
UTS
BamHI (7369)
NotI (5639)
TAG (5269)
HindIII (5619)

FIG. 4

Fragment A

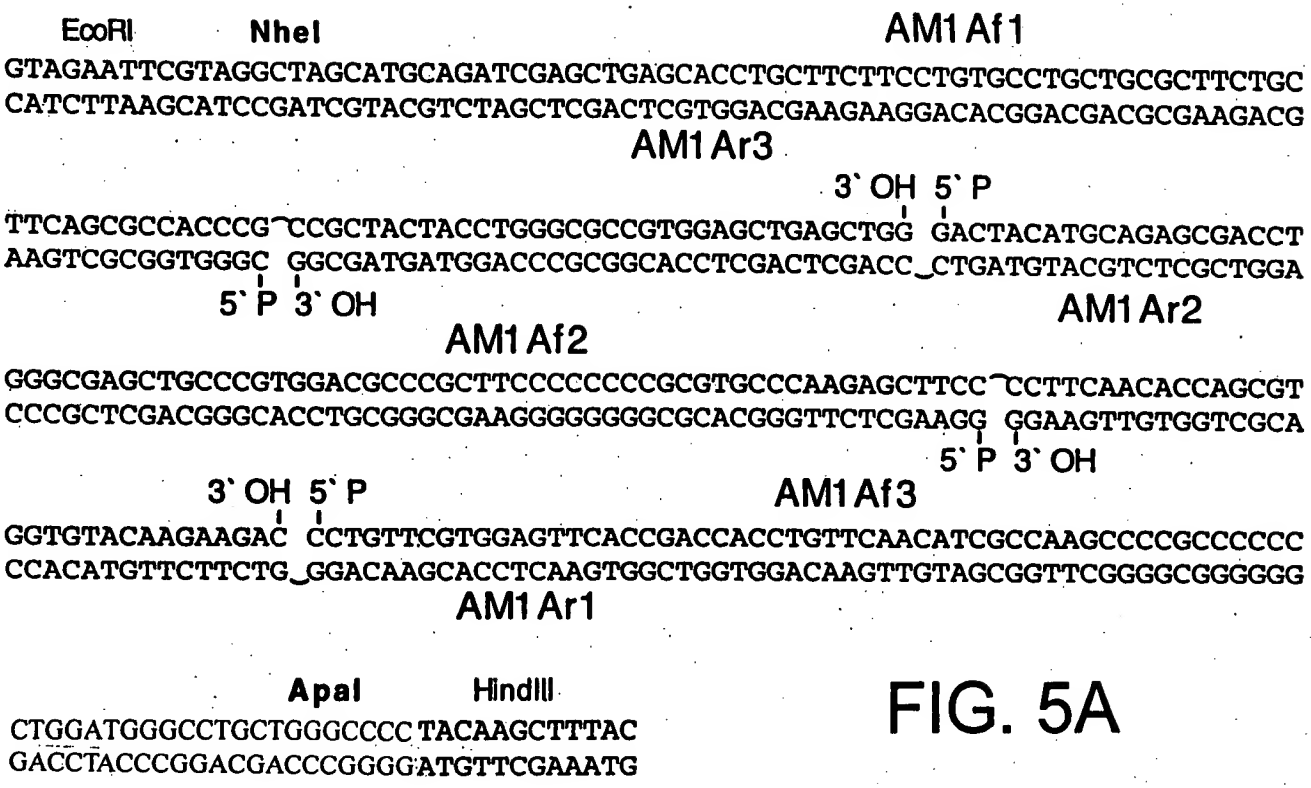


FIG. 5A

Fragment B

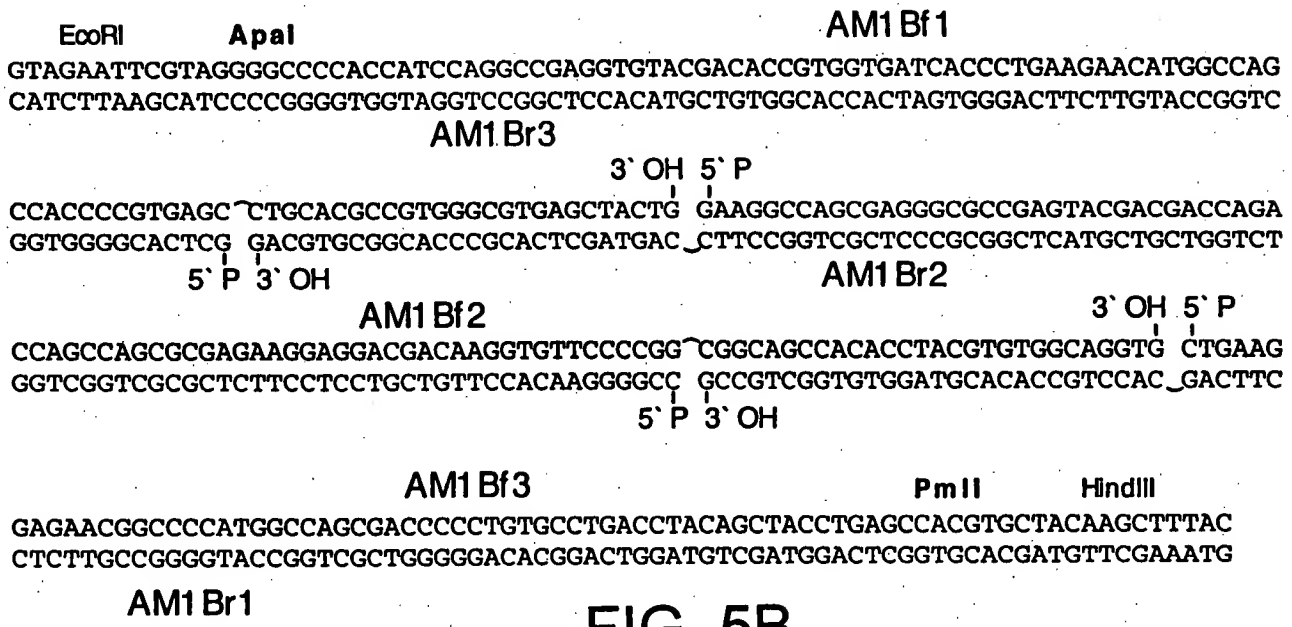


FIG. 5B

Fragment C

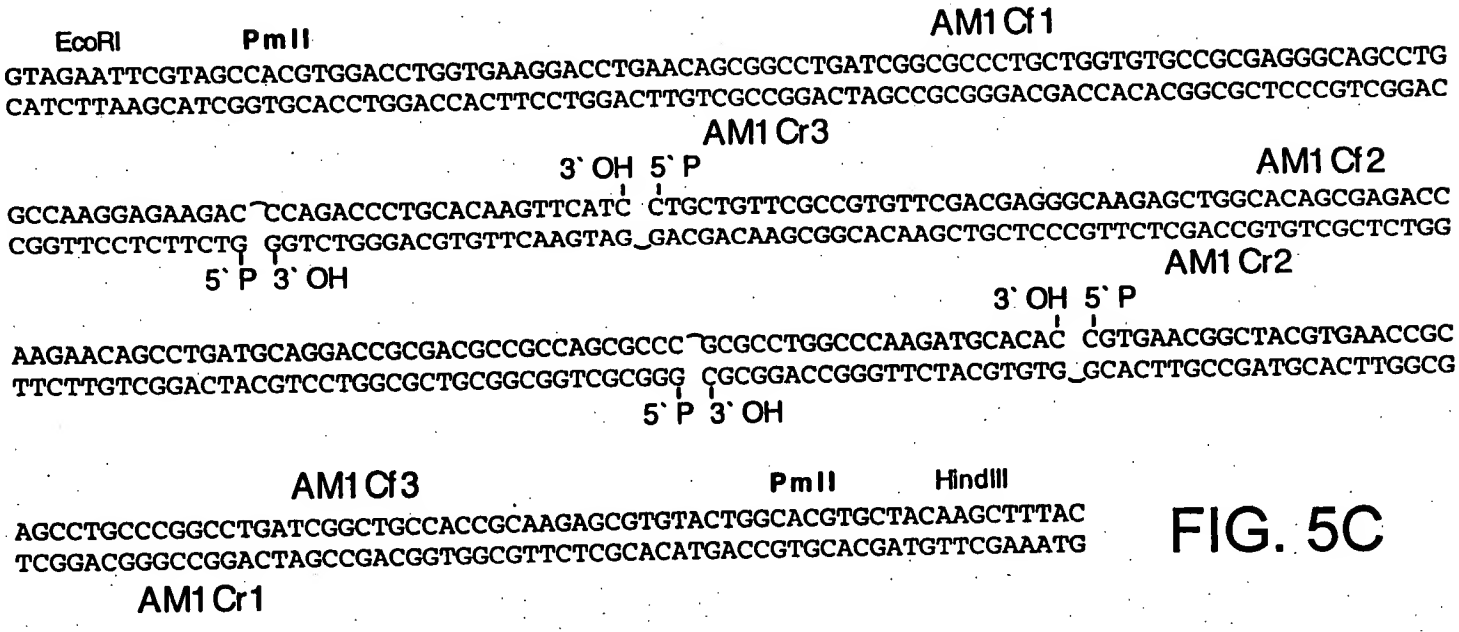


FIG. 5C

Fragment D

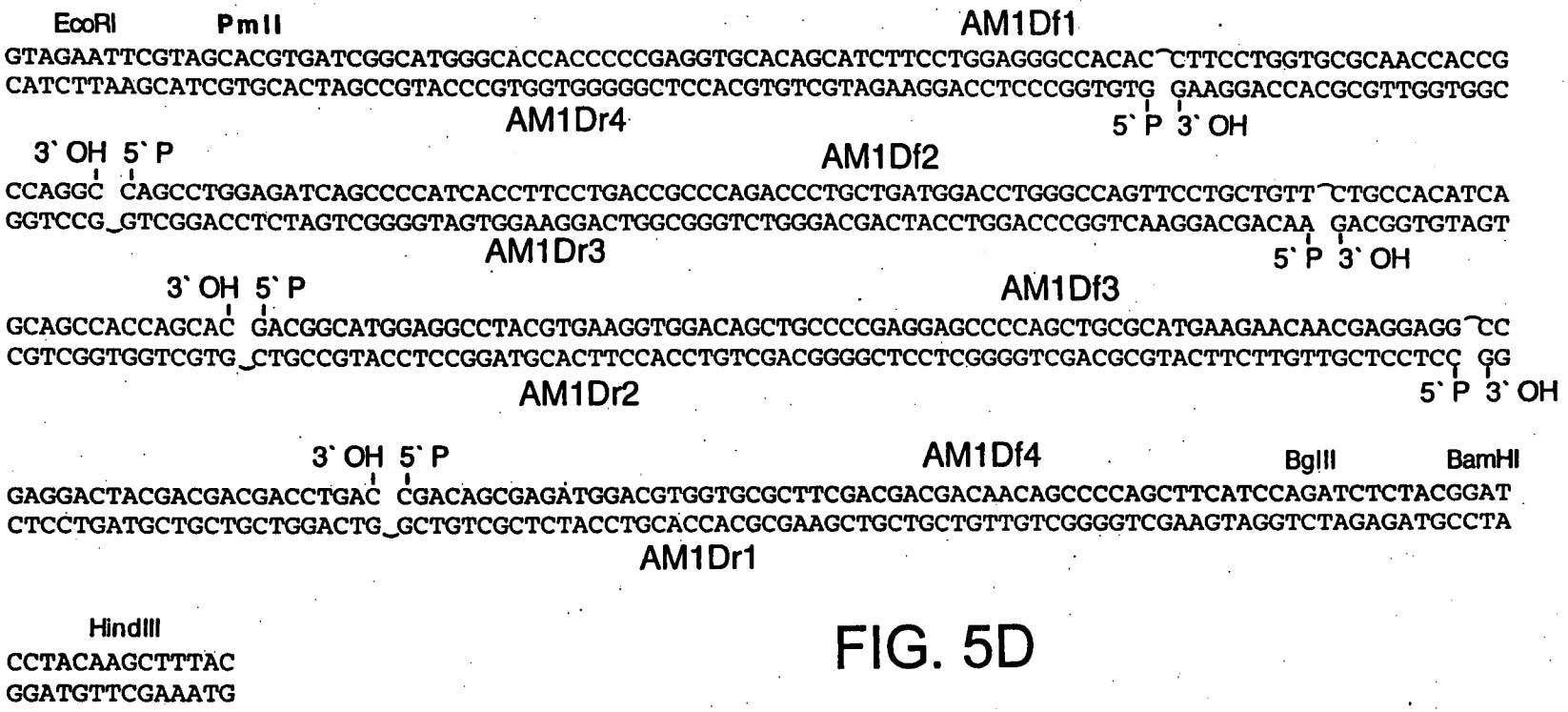


FIG. 5D

Fragment E

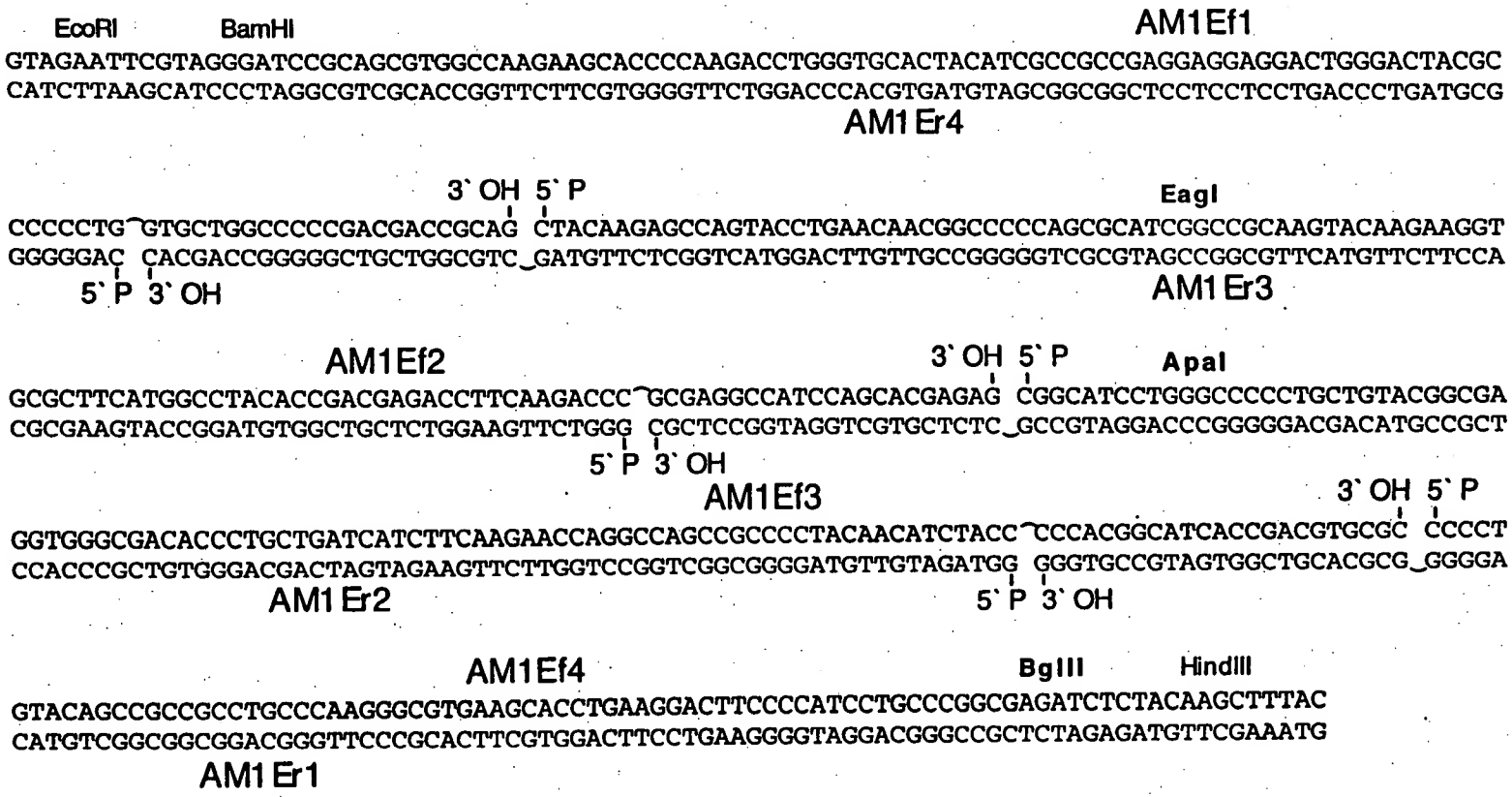


FIG. 5E

Fragment F

FIG. 5F

Fragment G

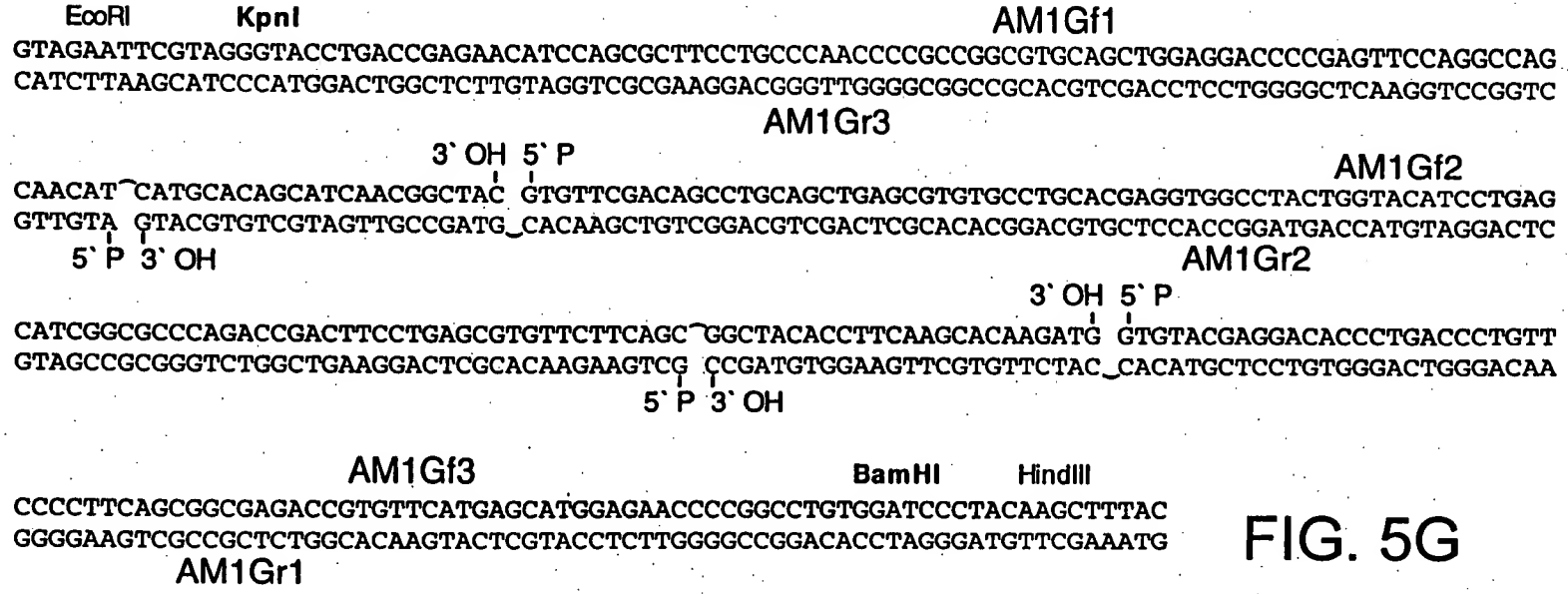


FIG. 5G

Fragment H

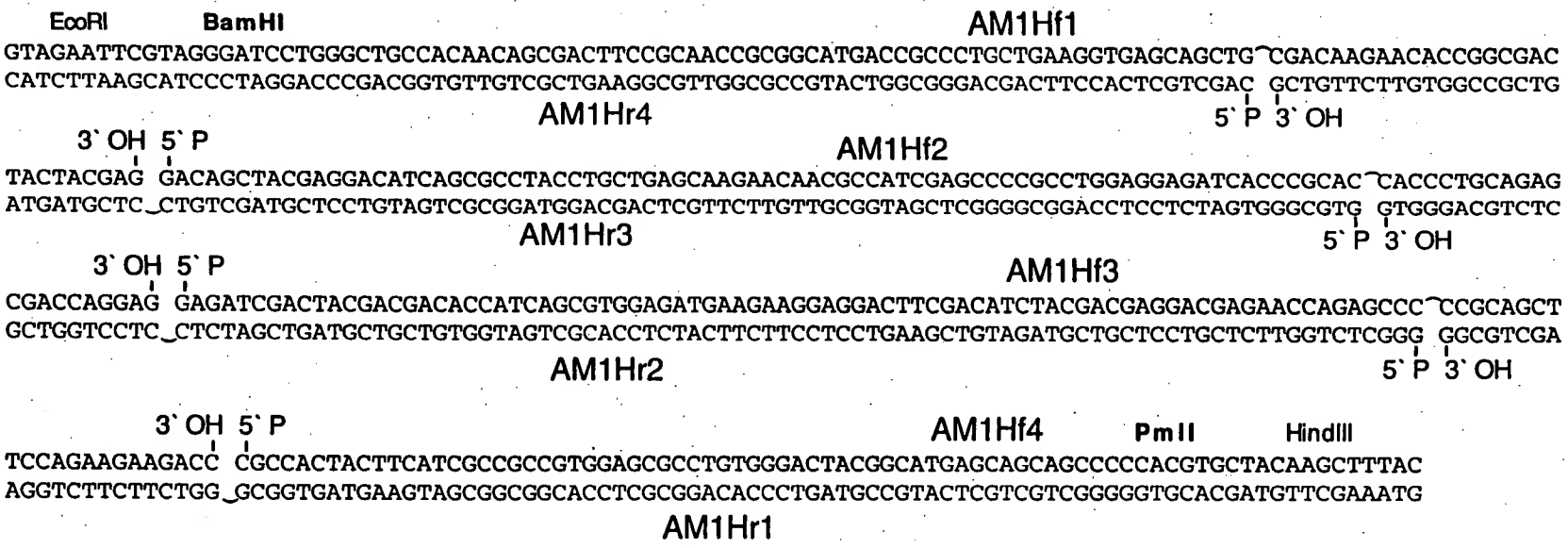


FIG. 5H

Fragment I

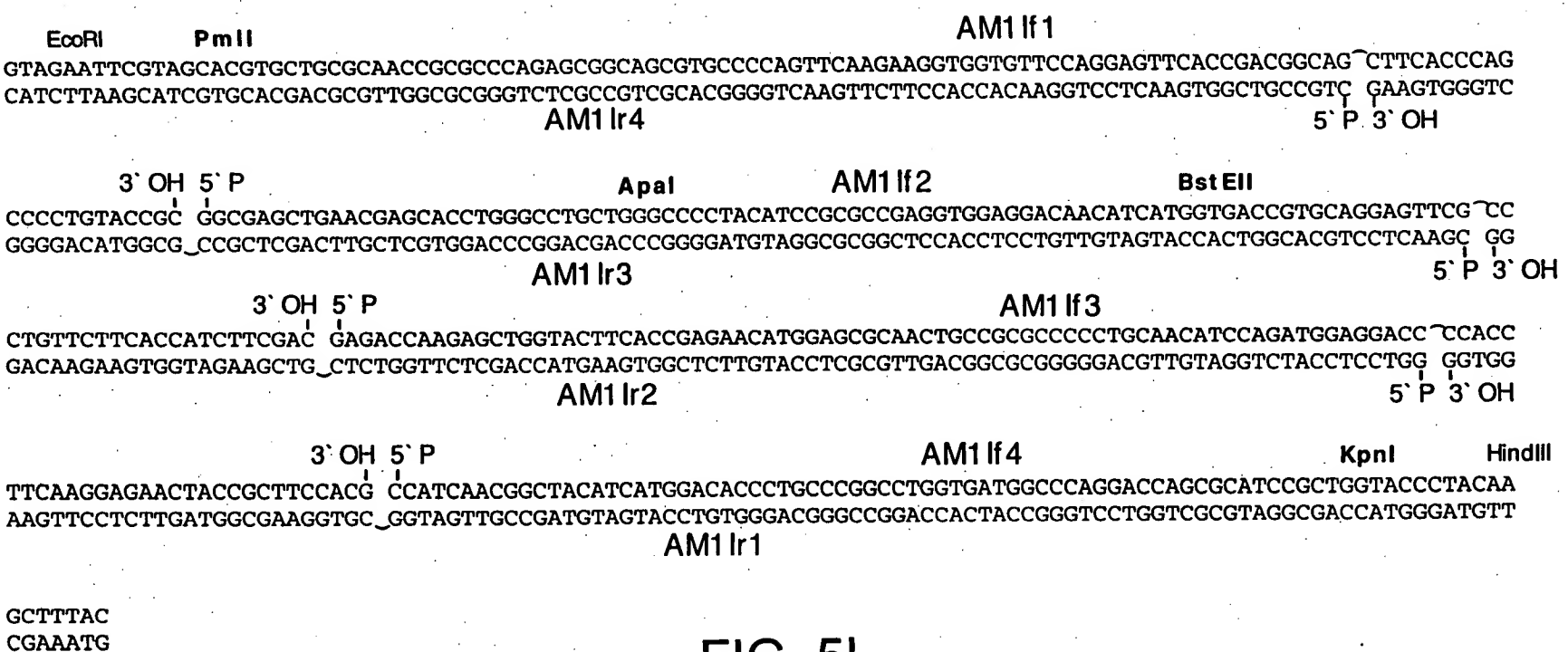


FIG. 5I

Fragment J

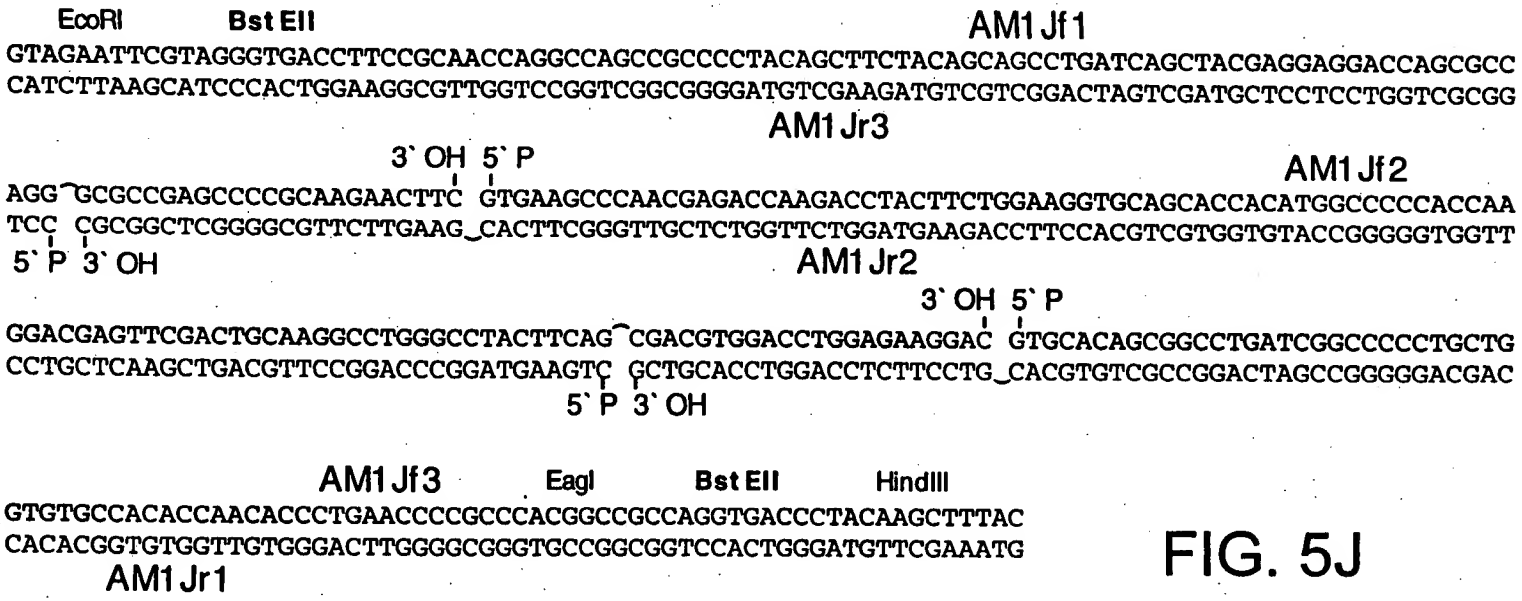


FIG. 5J

Fragment K

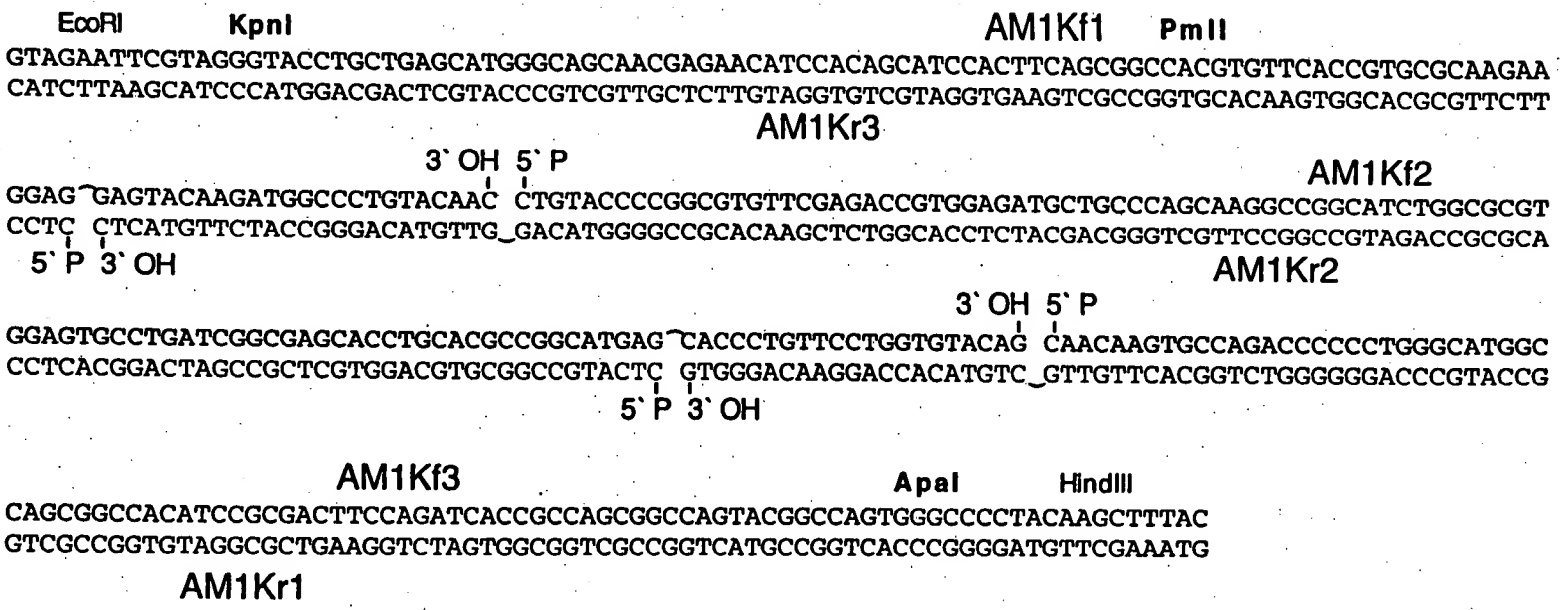


FIG. 5K

Fragment L

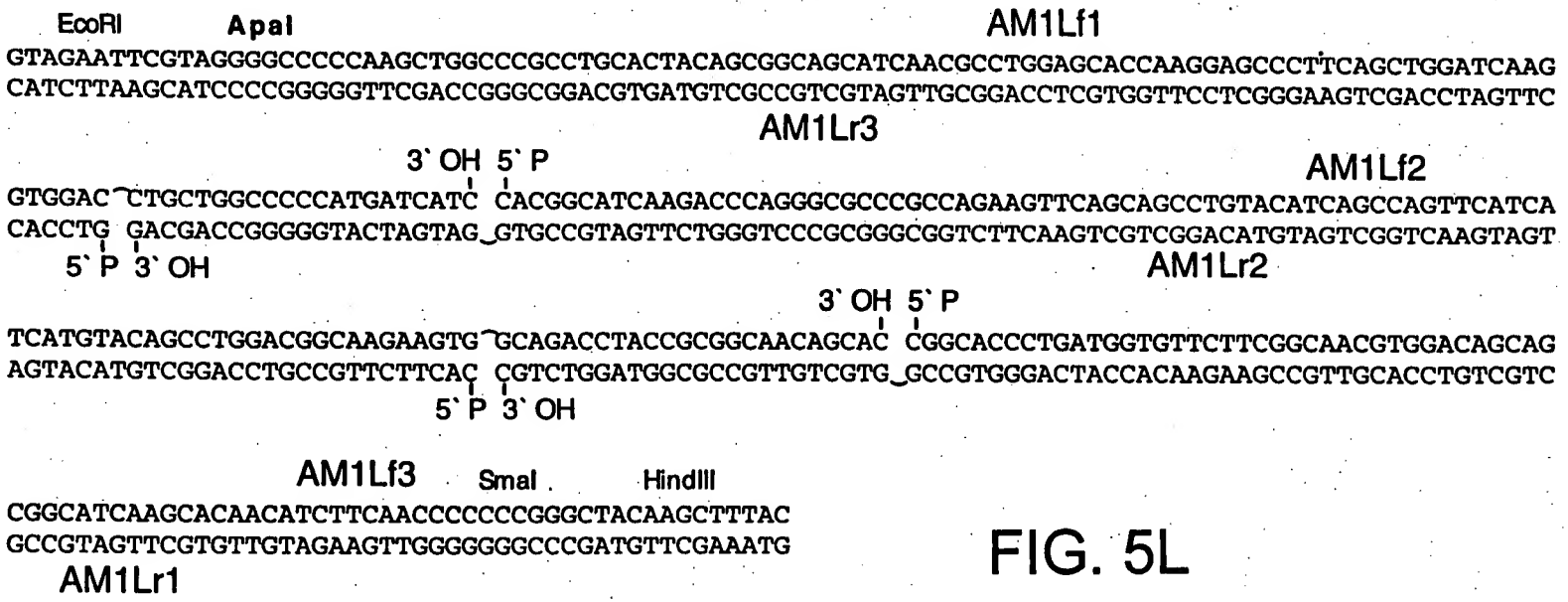


FIG. 5L

Fragment M

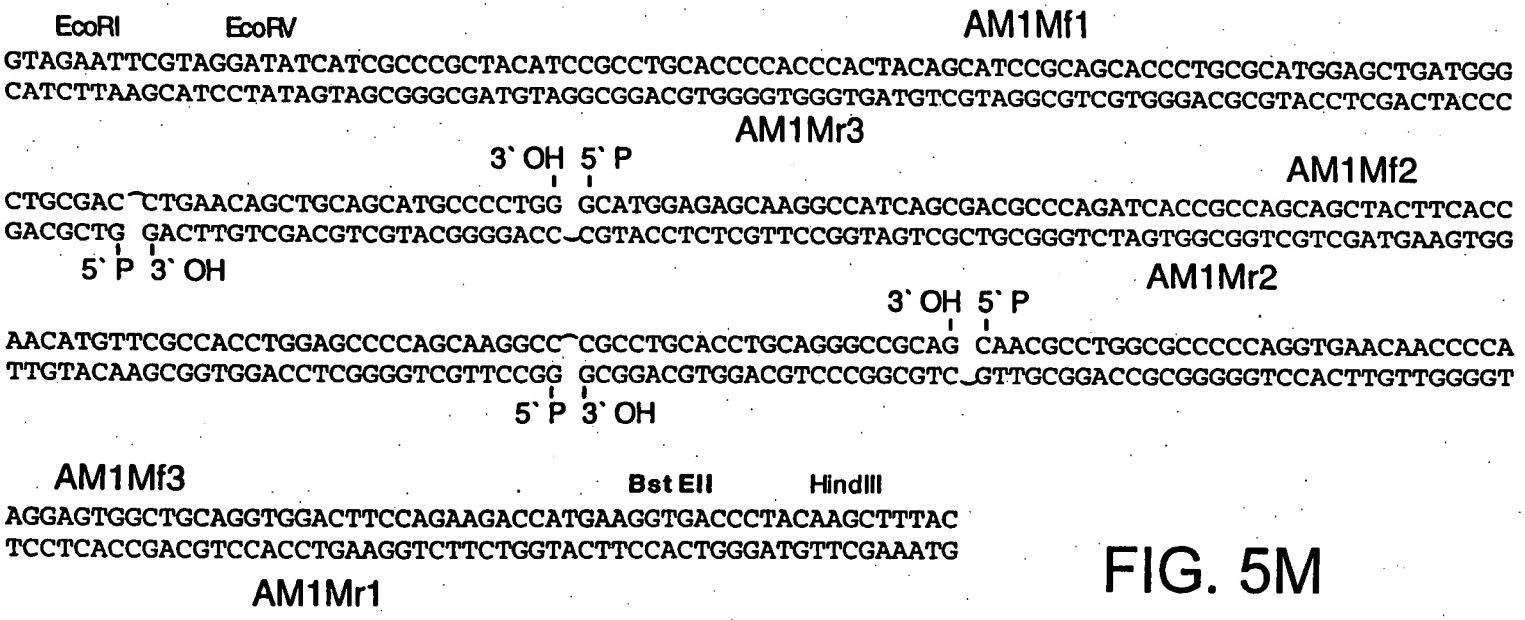


FIG. 5M

Fragment N

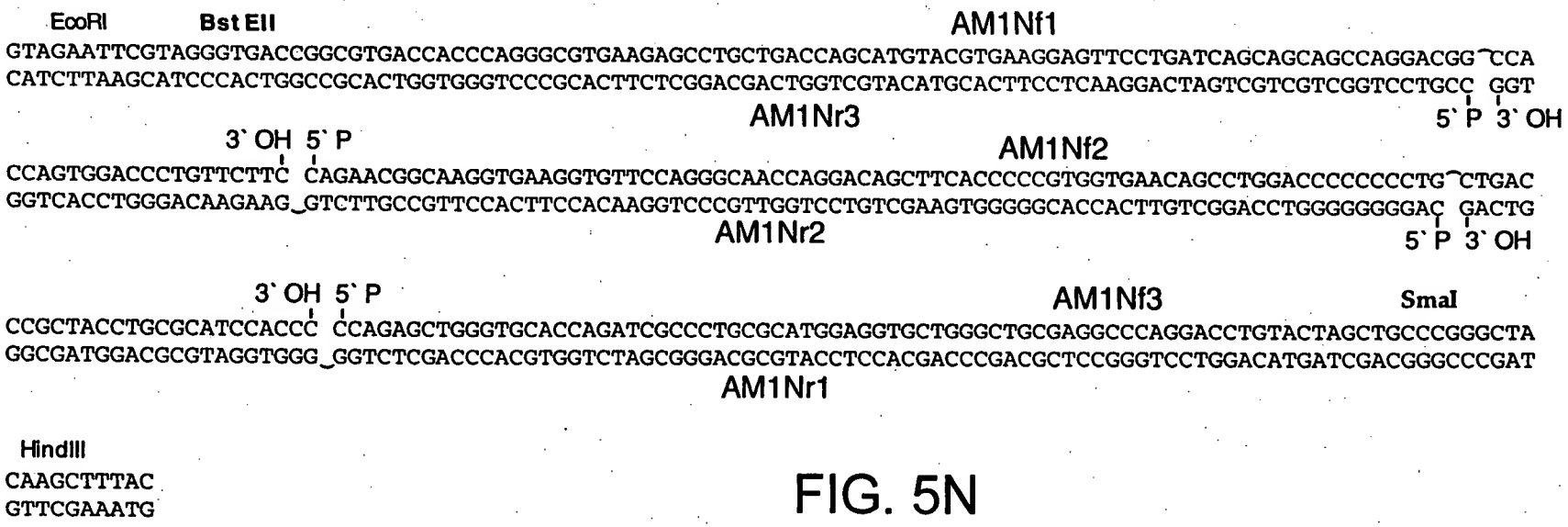


FIG. 5N

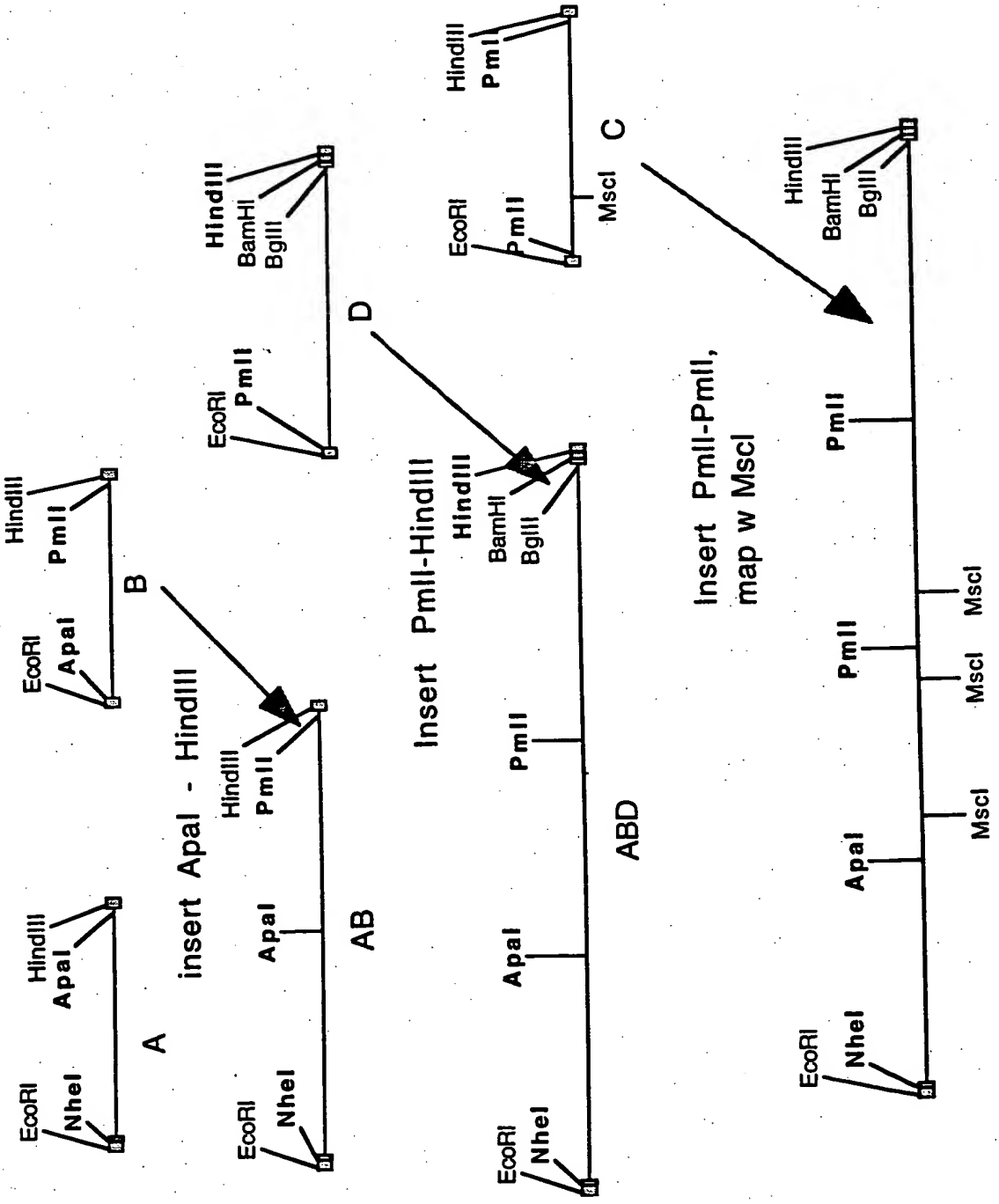

A
EcoRI
NheI
HindIII
ApaI
B
HindIII
PmlI
EcoRI
ApaI
insert ApaI - HindIII
HindIII
PmlI
ApaI
EcoRI
NheI
AB
D
HindIII
BamHI
BglII
EcoRI
PmlI
Insert PmlI-HindIII
HindIII
BamHI
BglII
PmlI
ApaI
EcoRI
NheI
ABD
C
HindIII
PmlI
EcoRI
PmlI
MscI
Insert PmlI-PmlI,
map w MscI
HindIII
BamHI
BglII
PmlI
PmlI
MscI
MscI
ApaI
MscI
EcoRI
NheI
ABCD

FIG. 6A

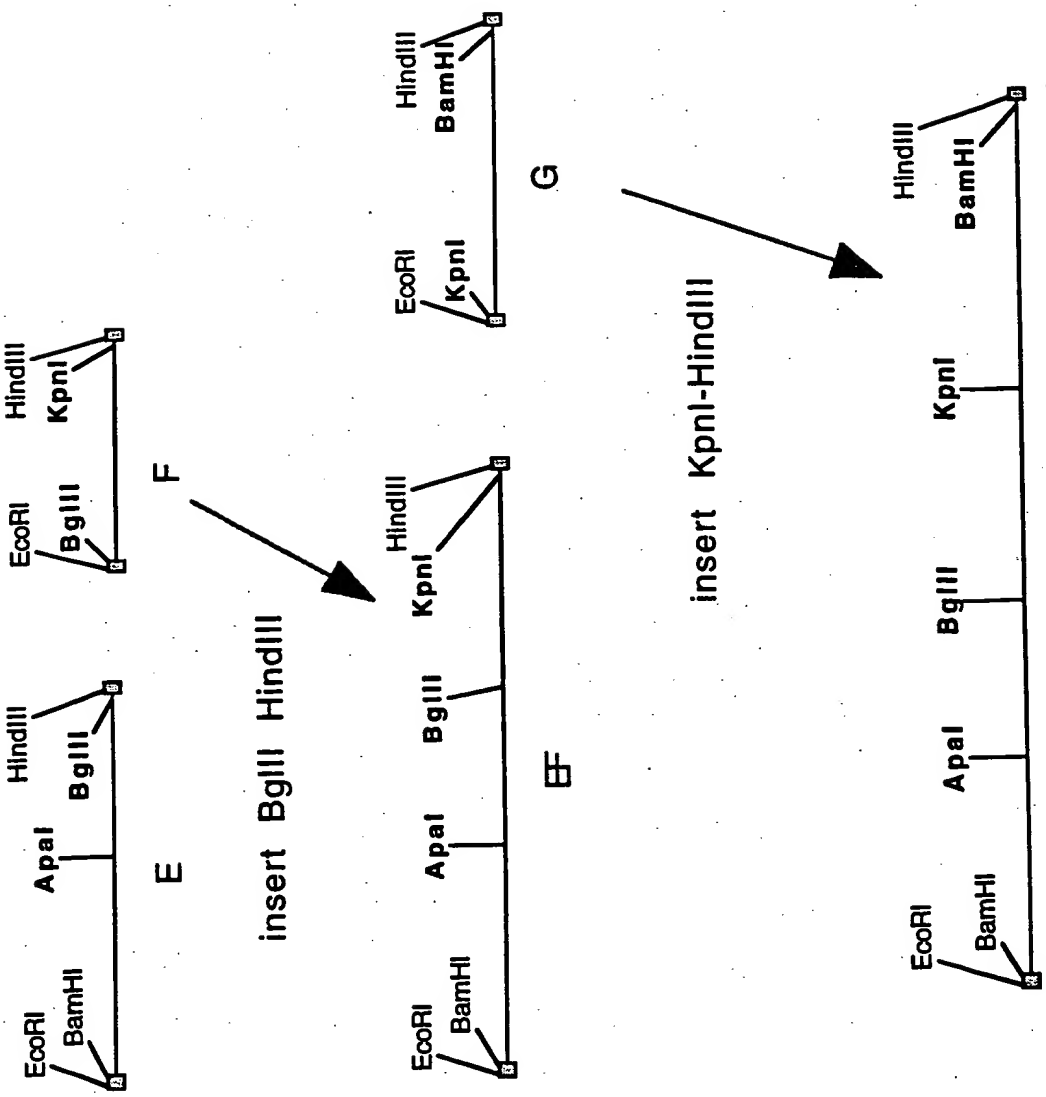

HindIII
KpnI
EcoRI
BglII
F
HindIII
BglII
ApaI
EcoRI
BamHI
E
insert BglII HindIII
HindIII
KpnI
BglII
ApaI
EcoRI
BamHI
EF
HindIII
BamHI
EcoRI
KpnI
G
insert KpnI-HindIII
HindIII
BamHI
KpnI
BglII
ApaI
EcoRI
BamHI
EFG

FIG. 6B

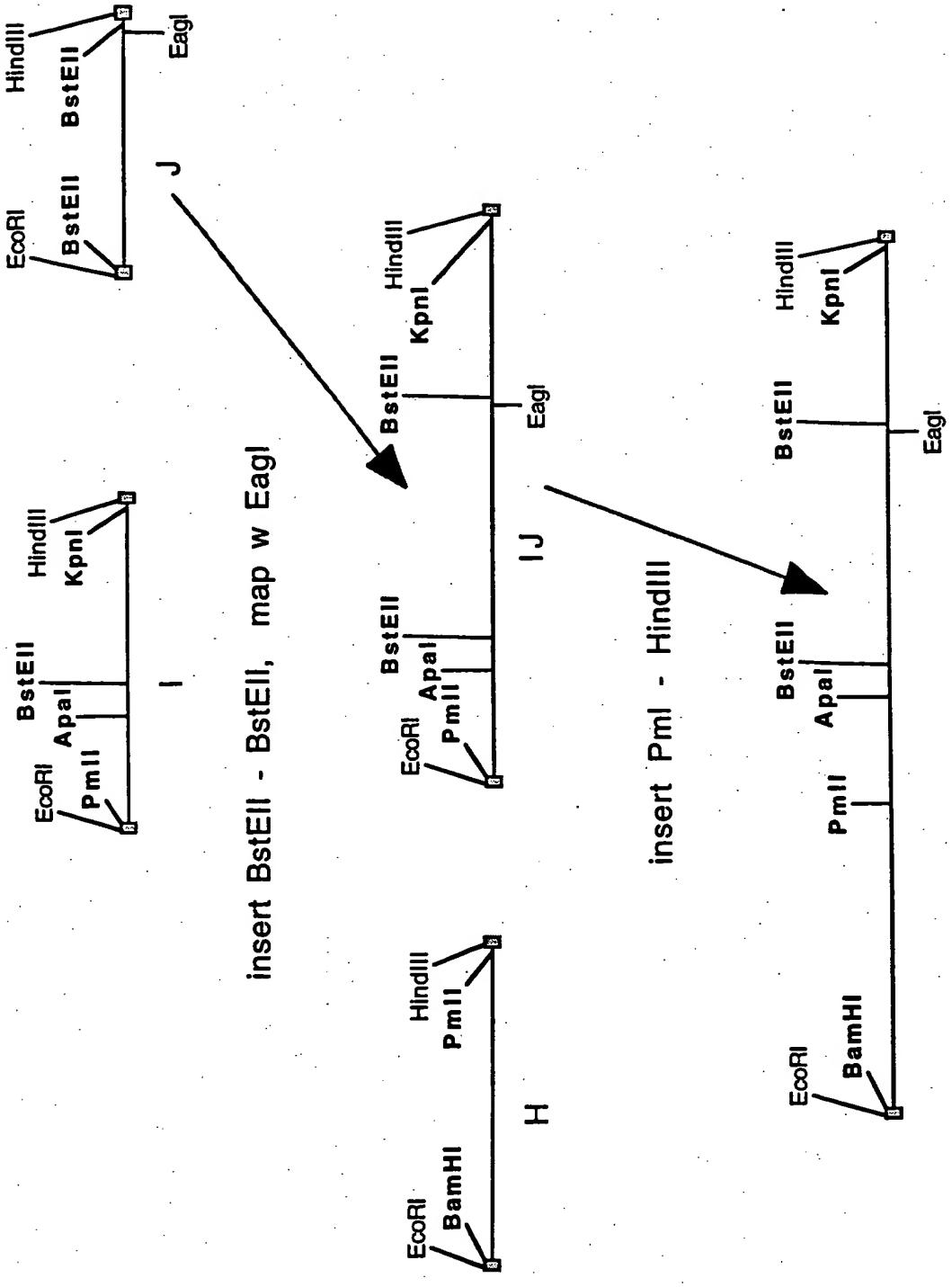

HindIII
BstEII
EagI
EcoRI
BstEII
J
HindIII
KpnI
BstEII
EagI
EcoRI
BstEII
ApaI
PmlI
IJ
HindIII
KpnI
BstEII
EagI
BstEII
ApaI
PmlI
HIJ
EcoRI
BamHI
H
HindIII
PmlI
EcoRI
BstEII
ApaI
PmlI
I
insert BstEII - BstEII, map w EagI
insert Pml - HindIII

FIG. 6C

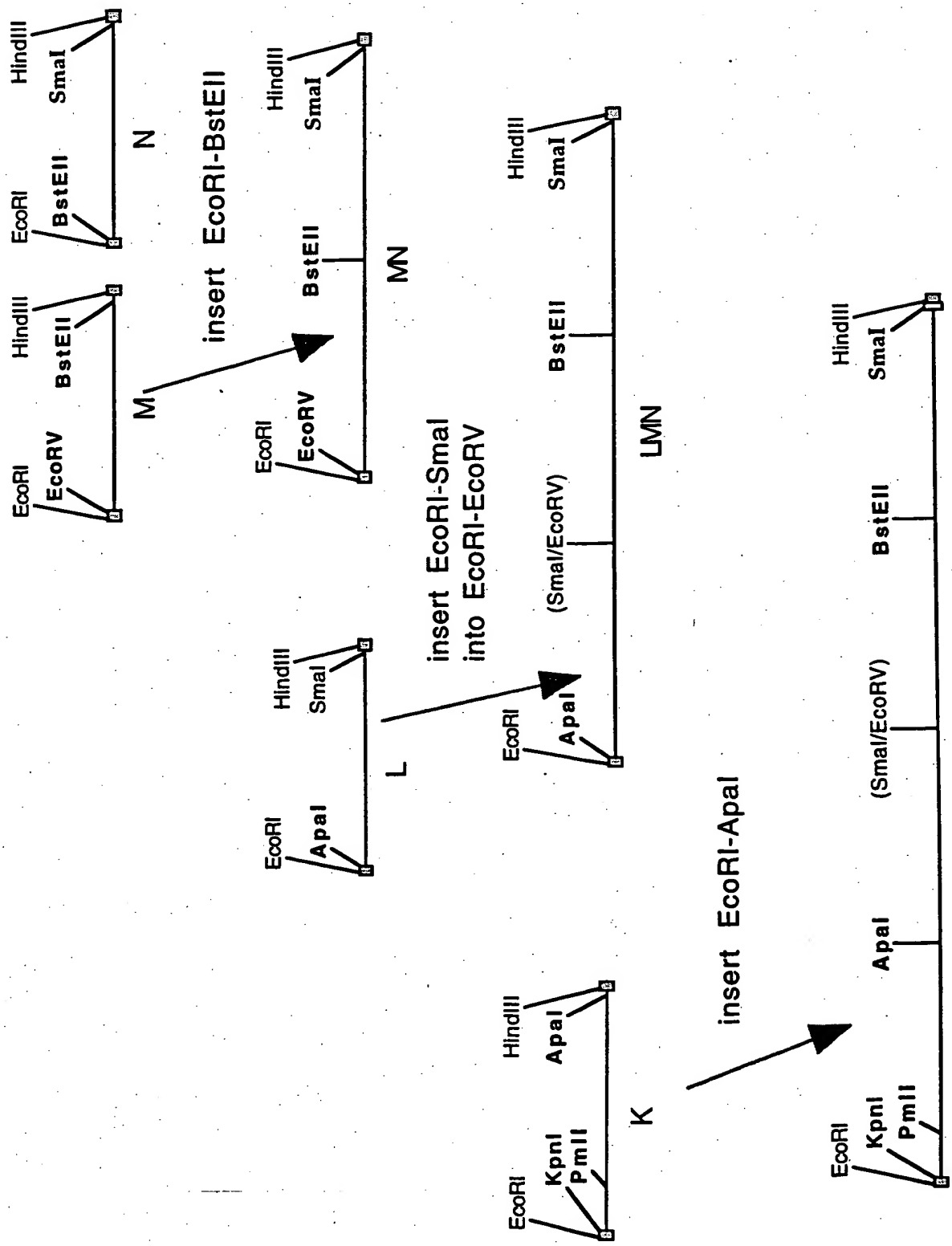

HindIII
SmaI
EcoRI
BstEII
N
HindIII
BstEII
EcoRV
EcoRI
M
insert EcoRI-BstEII
HindIII
SmaI
BstEII
MN
EcoRI
EcoRV
HindIII
SmaI
EcoRI
ApaI
L
insert EcoRI-SmaI
into EcoRI-EcoRV
HindIII
SmaI
BstEII
(SmaI/EcoRV)
LMN
EcoRI
ApaI
HindIII
ApaI
EcoRI
KpnI
PmlI
K
insert EcoRI-ApaI
HindIII
SmaI
BstEII
(SmaI/EcoRV)
ApaI
KLMN
EcoRI
KpnI
PmlI

FIG. 6D

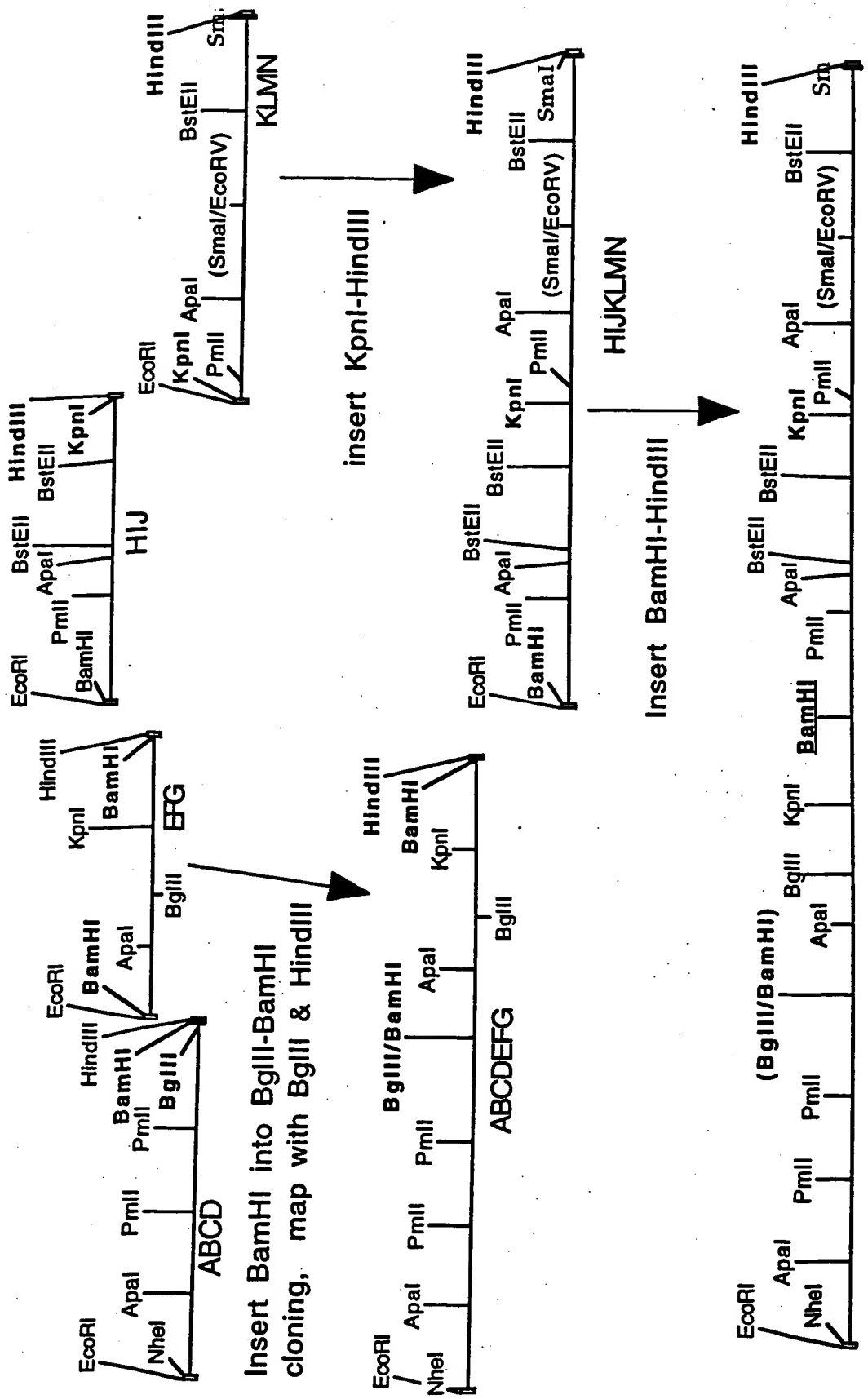

EcoRI
ApaI
NheI
PmlI
ABCD
BglII
BamHI
HindIII
EFG
Insert BamHI into BglII-BamHI
cloning, map with BglII & HindIII
ABCDEFG
BglII/BamHI
KpnI
HIJ
BstEII
(SmaI/EcoRV)
KLMN
insert KpnI-HindIII
HIJKLMN
Insert BamHI-HindIII
ABCDEFGHIJKLMN, i.e. pAM1-1

FIG. 6E

```
      EcoRI      NheI
   1  TAGAATTCGTAGGCTAGCATGCAGATCGAGCTGAGCACCTGCTTCTTCCTGTGCCTGCTGCGCTTCTGCTTC
                    1▶ MetGlnIleGluLeuSerThrCysPhePheLeuCysLeuLeuArgPheCysPhe
  73  AGCGCCACCCGCCGCTACTACCTGGGCGCCGTGGAGCTGAGCTGGGACTACATGCAGAGCGACCTGGGCGAG
  19▶ SerAlaThrArgArgTyrTyrLeuGlyAlaValGluLeuSerTrpAspTyrMetGlnSerAspLeuGlyGlu
 145  CTGCCCGTGGACGCCCGCTTCCCCCCCCGCGTGCCCAAGAGCTTCCCCTTCAACACCAGCGTGGTGTACAAG
  43▶ LeuProValAspAlaArgPheProProArgValProLysSerPheProPheAsnThrSerValValTyrLys
 217  AAGACCCTGTTCGTGGAGTTCACCGACCACCTGTTCAACATCGCCAAGCCCCGCCCCCCCTGGATGGGCCTG
  67▶ LysThrLeuPheValGluPheThrAspHisLeuPheAsnIleAlaLysProArgProProTrpMetGlyLeu
      ApaI                                                          MscI
 289  CTGGGCCCCACCATCCAGGCCGAGGTGTACGACACCGTGGTGATCACCCTGAAGAACATGGCCAGCCACCCC
  91▶ LeuGlyProThrIleGlnAlaGluValTyrAspThrValValIleThrLeuLysAsnMetAlaSerHisPro
 361  GTGAGCCTGCACGCCGTGGGCGTGAGCTACTGGAAGGCCAGCGAGGGCGCCGAGTACGACGACCAGACCAGC
 115▶ ValSerLeuHisAlaValGlyValSerTyrTrpLysAlaSerGluGlyAlaGluTyrAspAspGlnThrSer
 433  CAGCGCGAGAAGGAGGACGACAAGGTGTTCCCCGGCGGCAGCCACACCTACGTGTGGCAGGTGCTGAAGGAG
 139▶ GlnArgGluLysGluAspAspLysValPheProGlyGlySerHisThrTyrValTrpGlnValLeuLysGlu
          MscI                                                  PmlI
 505  AACGGCCCCATGGCCAGCGACCCCCTGTGCCTGACCTACAGCTACCTGAGCCACGTGGACCTGGTGAAGGAC
 163▶ AsnGlyProMetAlaSerAspProLeuCysLeuThrTyrSerTyrLeuSerHisValAspLeuValLysAsp
                                                          MscI
 577  CTGAACAGCGGCCTGATCGGCGCCCTGCTGGTGTGCCGCGAGGGCAGCCTGGCCAAGGAGAAGACCCAGACC
 187▶ LeuAsnSerGlyLeuIleGlyAlaLeuLeuValCysArgGluGlySerLeuAlaLysGluLysThrGlnThr
 649  CTGCACAAGTTCATCCTGCTGTTCGCCGTGTTCGACGAGGGCAAGAGCTGGCACAGCGAGACCAAGAACAGC
 211▶ LeuHisLysPheIleLeuLeuPheAlaValPheAspGluGlyLysSerTrpHisSerGluThrLysAsnSer
 721  CTGATGCAGGACCGCGACGCCGCCAGCGCCCGCGCCTGGCCCAAGATGCACACCGTGAACGGCTACGTGAAC
 235▶ LeuMetGlnAspArgAspAlaAlaSerAlaArgAlaTrpProLysMetHisThrValAsnGlyTyrValAsn
                                                          PmlI
 793  CGCAGCCTGCCCGGCCTGATCGGCTGCCACCGCAAGAGCGTGTACTGGCACGTGATCGGCATGGGCACCACC
 259▶ ArgSerLeuProGlyLeuIleGlyCysHisArgLysSerValTyrTrpHisValIleGlyMetGlyThrThr
 865  CCCGAGGTGCACAGCATCTTCCTGGAGGGCCACACCTTCCTGGTGCGCAACCACCGCCAGGCCAGCCTGGAG
 283▶ ProGluValHisSerIlePheLeuGluGlyHisThrPheLeuValArgAsnHisArgGlnAlaSerLeuGlu
 937  ATCAGCCCCATCACCTTCCTGACCGCCCAGACCCTGCTGATGGACCTGGGCCAGTTCCTGCTGTTCTGCCAC
 307▶ IleSerProIleThrPheLeuThrAlaGlnThrLeuLeuMetAspLeuGlyGlnPheLeuLeuPheCysHis
1009  ATCAGCAGCCACCAGCACGACGGCATGGAGGCCTACGTGAAGGTGGACAGCTGCCCCGAGGAGCCCCAGCTG
 331▶ IleSerSerHisGlnHisAspGlyMetGluAlaTyrValLysValAspSerCysProGluGluProGlnLeu
1081  CGCATGAAGAACAACGAGGAGGCCGAGGACTACGACGACGACCTGACCGACAGCGAGATGGACGTGGTGCGC
 355▶ ArgMetLysAsnAsnGluGluAlaGluAspTyrAspAspAspLeuThrAspSerGluMetAspValValArg
                                    (BglII/BamHI)
1153  TTCGACGACGACAACAGCCCCAGCTTCATCCAGATCCGCAGCGTGGCCAAGAAGCACCCCAAGACCTGGGTG
 379▶ PheAspAspAspAsnSerProSerPheIleGlnIleArgSerValAlaLysLysHisProLysThrTrpVal
1225  CACTACATCGCCGCCGAGGAGGAGGACTGGGACTACGCCCCCCTGGTGCTGGCCCCCGACGACCGCAGCTAC
 403▶ HisTyrIleAlaAlaGluGluGluAspTrpAspTyrAlaProLeuValLeuAlaProAspAspArgSerTyr
                                        EagI
1297  AAGAGCCAGTACCTGAACAACGGCCCCCAGCGCATCGGCCGCAAGTACAAGAAGGTGCGCTTCATGGCCTAC
 427▶ LysSerGlnTyrLeuAsnAsnGlyProGlnArgIleGlyArgLysTyrLysLysValArgPheMetAlaTyr
                                                          ApaI
1369  ACCGACGAGACCTTCAAGACCCGCGAGGCCATCCAGCACGAGAGCGGCATCCTGGGCCCCCTGCTGTACGGC
 451▶ ThrAspGluThrPheLysThrArgGluAlaIleGlnHisGluSerGlyIleLeuGlyProLeuLeuTyrGly
```

FIG. 7A

```
1441 GAGGTGGGCGACACCCTGCTGATCATCTTCAAGAACCAGGCCAGCCGCCCCTACAACATCTACCCCCACGGC
 475▶GluValGlyAspThrLeuLeuIleIlePheLysAsnGlnAlaSerArgProTyrAsnIleTyrProHisGly
1513 ATCACCGACGTGCGCCCCCTGTACAGCCGCCGCCTGCCCAAGGGCGTGAAGCACCTGAAGGACTTCCCCATC
 499▶IleThrAspValArgProLeuTyrSerArgArgLeuProLysGlyValLysHisLeuLysAspPheProIle
                BglII
1585 CTGCCCGGCGAGATCTTCAAGTACAAGTGGACCGTGACCGTGGAGGACGGCCCCACCAAGAGCGACCCCCGC
 523▶LeuProGlyGluIlePheLysTyrLysTrpThrValThrValGluAspGlyProThrLysSerAspProArg
1657 TGCCTGACCCGCTACTACAGCAGCTTCGTGAACATGGAGCGCGACCTGGCCAGCGGCCTGATCGGCCCCCTG
 547▶CysLeuThrArgTyrTyrSerSerPheValAsnMetGluArgAspLeuAlaSerGlyLeuIleGlyProLeu
1729 CTGATCTGCTACAAGGAGAGCGTGGACCAGCGCGGCAACCAGATCATGAGCGACAAGCGCAACGTGATCCTG
 571▶LeuIleCysTyrLysGluSerValAspGlnArgGlyAsnGlnIleMetSerAspLysArgAsnValIleLeu
                      KpnI
1801 TTCAGCGTGTTCGACGAGAACCGCAGCTGGTACCTGACCGAGAACATCCAGCGCTTCCTGCCCAACCCCGCC
 595▶PheSerValPheAspGluAsnArgSerTrpTyrLeuThrGluAsnIleGlnArgPheLeuProAsnProAla
1873 GGCGTGCAGCTGGAGGACCCCGAGTTCCAGGCCAGCAACATCATGCACAGCATCAACGGCTACGTGTTCGAC
 619▶GlyValGlnLeuGluAspProGluPheGlnAlaSerAsnIleMetHisSerIleAsnGlyTyrValPheAsp
1945 AGCCTGCAGCTGAGCGTGTGCCTGCACGAGGTGGCCTACTGGTACATCCTGAGCATCGGCGCCCAGACCGAC
 643▶SerLeuGlnLeuSerValCysLeuHisGluValAlaTyrTrpTyrIleLeuSerIleGlyAlaGlnThrAsp
2017 TTCCTGAGCGTGTTCTTCAGCGGCTACACCTTCAAGCACAAGATGGTGTACGAGGACACCCTGACCCTGTTC
 667▶PheLeuSerValPhePheSerGlyTyrThrPheLysHisLysMetValTyrGluAspThrLeuThrLeuPhe
                                  BamHI
2089 CCCTTCAGCGGCGAGACCGTGTTCATGAGCATGGAGAACCCCGGCCTGTGGATCCTGGGCTGCCACAACAGC
 691▶ProPheSerGlyGluThrValPheMetSerMetGluAsnProGlyLeuTrpIleLeuGlyCysHisAsnSer
2161 GACTTCCGCAACCGCGGCATGACCGCCCTGCTGAAGGTGAGCAGCTGCGACAAGAACACCGGCGACTACTAC
 715▶AspPheArgAsnArgGlyMetThrAlaLeuLeuLysValSerSerCysAspLysAsnThrGlyAspTyrTyr
2233 GAGGACAGCTACGAGGACATCAGCGCCTACCTGCTGAGCAAGAACAACGCCATCGAGCCCCGCCTGGAGGAG
 739▶GluAspSerTyrGluAspIleSerAlaTyrLeuLeuSerLysAsnAsnAlaIleGluProArgLeuGluGlu
                                        BstXI
2305 ATCACCCGCACCACCCTGCAGAGCGACCAGGAGGAGATCGACTACGACGACACCATCAGCGTGGAGATGAAG
 763▶IleThrArgThrThrLeuGlnSerAspGlnGluGluIleAspTyrAspAspThrIleSerValGluMetLys
2377 AAGGAGGACTTCGACATCTACGACGAGGACGAGAACCAGAGCCCCCGCAGCTTCCAGAAGAAGACCCGCCAC
 787▶LysGluAspPheAspIleTyrAspGluAspGluAsnGlnSerProArgSerPheGlnLysLysThrArgHis
                                              PmlI
2449 TACTTCATCGCCGCCGTGGAGCGCCTGTGGGACTACGGCATGAGCAGCAGCCCCCACGTGCTGCGCAACCGC
 811▶TyrPheIleAlaAlaValGluArgLeuTrpAspTyrGlyMetSerSerSerProHisValLeuArgAsnArg
2521 GCCCAGAGCGGCAGCGTGCCCCAGTTCAAGAAGGTGGTGTTCCAGGAGTTCACCGACGGCAGCTTCACCCAG
 835▶AlaGlnSerGlySerValProGlnPheLysLysValValPheGlnGluPheThrAspGlySerPheThrGln
                                                    ApaI
2593 CCCCTGTACCGCGGCGAGCTGAACGAGCACCTGGGCCTGCTGGGCCCCTACATCCGCGCCGAGGTGGAGGAC
 859▶ProLeuTyrArgGlyGluLeuAsnGluHisLeuGlyLeuLeuGlyProTyrIleArgAlaGluValGluAsp
            BstEII
2665 AACATCATGGTGACCTTCCGCAACCAGGCCAGCCGCCCCTACAGCTTCTACAGCAGCCTGATCAGCTACGAG
 883▶AsnIleMetValThrPheArgAsnGlnAlaSerArgProTyrSerPheTyrSerSerLeuIleSerTyrGlu
2737 GAGGACCAGCGCCAGGGCGCCGAGCCCCGCAAGAACTTCGTGAAGCCCAACGAGACCAAGACCTACTTCTGG
 907▶GluAspGlnArgGlnGlyAlaGluProArgLysAsnPheValLysProAsnGluThrLysThrTyrPheTrp
2809 AAGGTGCAGCACCACATGGCCCCCACCAAGGACGAGTTCGACTGCAAGGCCTGGGCCTACTTCAGCGACGTG
 931▶LysValGlnHisHisMetAlaProThrLysAspGluPheAspCysLysAlaTrpAlaTyrPheSerAspVal
```

FIG. 7B

```
2881 GACCTGGAGAAGGACGTGCACAGCGGCCTGATCGGCCCCCTGCTGGTGTGCCACACCAACACCCTGAACCCC
 955▶AspLeuGluLysAspValHisSerGlyLeuIleGlyProLeuLeuValCysHisThrAsnThrLeuAsnPro
          EagI      BstEII
2953 GCCCACGGCCGCCAGGTGACCGTGCAGGAGTTCGCCCTGTTCTTCACCATCTTCGACGAGACCAAGAGCTGG
 979▶AlaHisGlyArgGlnValThrValGlnGluPheAlaLeuPhePheThrIlePheAspGluThrLysSerTrp
3025 TACTTCACCGAGAACATGGAGCGCAACTGCCGCGCCCCCTGCAACATCCAGATGGAGGACCCCACCTTCAAG
1003▶TyrPheThrGluAsnMetGluArgAsnCysArgAlaProCysAsnIleGlnMetGluAspProThrPheLys
3097 GAGAACTACCGCTTCCACGCCATCAACGGCTACATCATGGACACCCTGCCCGGCCTGGTGATGGCCCAGGAC
1027▶GluAsnTyrArgPheHisAlaIleAsnGlyTyrIleMetAspThrLeuProGlyLeuValMetAlaGlnAsp
                 KpnI                                                    PmlI
3169 CAGCGCATCCGCTGGTACCTGCTGAGCATGGGCAGCAACGAGAACATCCACAGCATCCACTTCAGCGGCCAC
1051▶GlnArgIleArgTrpTyrLeuLeuSerMetGlySerAsnGluAsnIleHisSerIleHisPheSerGlyHis
3241 GTGTTCACCGTGCGCAAGAAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCCGGCGTGTTCGAGACC
1075▶ValPheThrValArgLysLysGluGluTyrLysMetAlaLeuTyrAsnLeuTyrProGlyValPheGluThr
3313 GTGGAGATGCTGCCCAGCAAGGCCGGCATCTGGCGCGTGGAGTGCCTGATCGGCGAGCACCTGCACGCCGGC
1099▶ValGluMetLeuProSerLysAlaGlyIleTrpArgValGluCysLeuIleGlyGluHisLeuHisAlaGly
3385 ATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCAGCGGCCACATCCGC
1123▶MetSerThrLeuPheLeuValTyrSerAsnLysCysGlnThrProLeuGlyMetAlaSerGlyHisIleArg
                                  ApaI
3457 GACTTCCAGATCACCGCCAGCGGCCAGTACGGCCAGTGGGCCCCCAAGCTGGCCCGCCTGCACTACAGCGGC
1147▶AspPheGlnIleThrAlaSerGlyGlnTyrGlyGlnTrpAlaProLysLeuAlaArgLeuHisTyrSerGly
3529 AGCATCAACGCCTGGAGCACCAAGGAGCCCTTCAGCTGGATCAAGGTGGACCTGCTGGCCCCCATGATCATC
1171▶SerIleAsnAlaTrpSerThrLysGluProPheSerTrpIleLysValAspLeuLeuAlaProMetIleIle
3601 CACGGCATCAAGACCCAGGGCGCCCGCCAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATCATGTAC
1195▶HisGlyIleLysThrGlnGlyAlaArgGlnLysPheSerSerLeuTyrIleSerGlnPheIleIleMetTyr
3673 AGCCTGGACGGCAAGAAGTGGCAGACCTACCGCGGCAACAGCACCGGCACCCTGATGGTGTTCTTCGGCAAC
1219▶SerLeuAspGlyLysLysTrpGlnThrTyrArgGlyAsnSerThrGlyThrLeuMetValPhePheGlyAsn
                                        (SmaI/EcoRV)
3745 GTGGACAGCAGCGGCATCAAGCACAACATCTTCAACCCCCCCATCATCGCCCGCTACATCCGCCTGCACCCC
1243▶ValAspSerSerGlyIleLysHisAsnIlePheAsnProProIleIleAlaArgTyrIleArgLeuHisPro
3817 ACCCACTACAGCATCCGCAGCACCCTGCGCATGGAGCTGATGGGCTGCGACCTGAACAGCTGCAGCATGCCC
1267▶ThrHisTyrSerIleArgSerThrLeuArgMetGluLeuMetGlyCysAspLeuAsnSerCysSerMetPro
3889 CTGGGCATGGAGAGCAAGGCCATCAGCGACGCCCAGATCACCGCCAGCAGCTACTTCACCAACATGTTCGCC
1291▶LeuGlyMetGluSerLysAlaIleSerAspAlaGlnIleThrAlaSerSerTyrPheThrAsnMetPheAla
3961 ACCTGGAGCCCCAGCAAGGCCCGCCTGCACCTGCAGGGCCGCAGCAACGCCTGGCGCCCCCAGGTGAACAAC
1315▶ThrTrpSerProSerLysAlaArgLeuHisLeuGlnGlyArgSerAsnAlaTrpArgProGlnValAsnAsn
                                              BstEII
4033 CCCAAGGAGTGGCTGCAGGTGGACTTCCAGAAGACCATGAAGGTGACCGGCGTGACCACCCAGGGCGTGAAG
1339▶ProLysGluTrpLeuGlnValAspPheGlnLysThrMetLysValThrGlyValThrThrGlnGlyValLys
4105 AGCCTGCTGACCAGCATGTACGTGAAGGAGTTCCTGATCAGCAGCAGCCAGGACGGCCACCAGTGGACCCTG
1363▶SerLeuLeuThrSerMetTyrValLysGluPheLeuIleSerSerSerGlnAspGlyHisGlnTrpThrLeu
4177 TTCTTCCAGAACGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCTTCACCCCCGTGGTGAACAGCCTG
1387▶PhePheGlnAsnGlyLysValLysValPheGlnGlyAsnGlnAspSerPheThrProValValAsnSerLeu
4249 GACCCCCCCCTGCTGACCCGCTACCTGCGCATCCACCCCCAGAGCTGGGTGCACCAGATCGCCCTGCGCATG
1411▶AspProProLeuLeuThrArgTyrLeuArgIleHisProGlnSerTrpValHisGlnIleAlaLeuArgMet
                                              SmaI      HindIII
4321 GAGGTGCTGGGCTGCGAGGCCCAGGACCTGTACTAGCTGCCCGGGCTACAAGCTTT
1435▶GluValLeuGlyCysGluAlaGlnAspLeuTyr•••
```

FIG. 7C

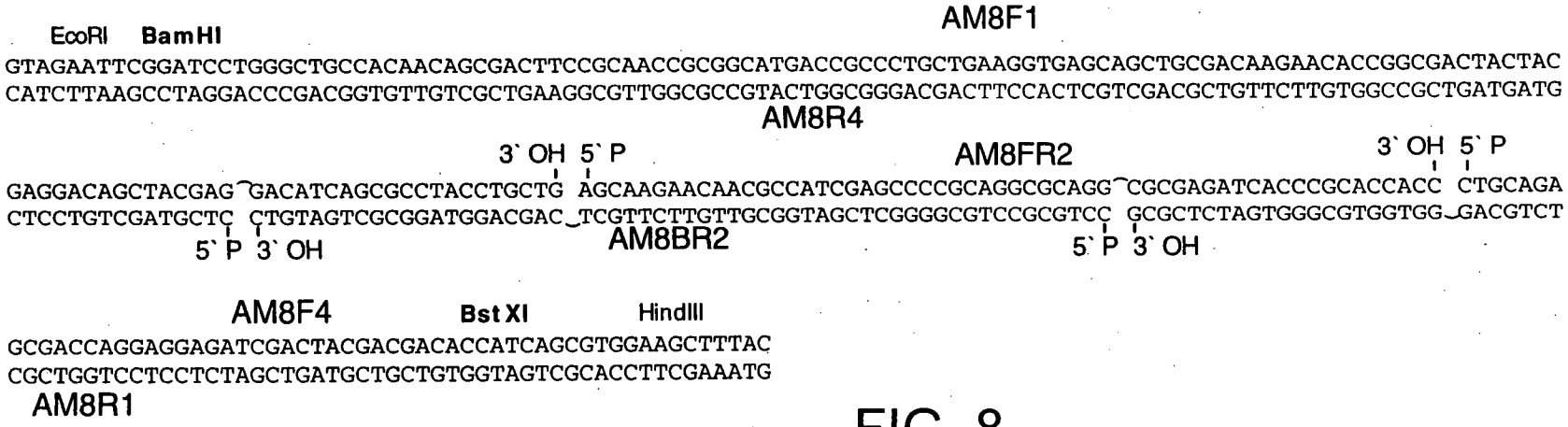

EcoRI
BamHI
AM8F1
GTAGAATTCGGATCCTGGGCTGCCACAACAGCGACTTCCGCAACCGCGGCATGACCGCCCTGCTGAAGGTGAGCAGCTGCGACAAGAACACCGGCGACTACTAC
CATCTTAAGCCTAGGACCCGACGGTGTTGTCGCTGAAGGCGTTGGCGCCGTACTGGCGGGACGACTTCCACTCGTCGACGCTGTTCTTGTGGCCGCTGATGATG
AM8R4
3` OH 5` P
AM8FR2
3` OH 5` P
GAGGACAGCTACGAG GACATCAGCGCCTACCTGCTG AGCAAGAACAACGCCATCGAGCCCCGCAGGCGCAGG CGCGAGATCACCCGCACCACC CTGCAGA
CTCCTGTCGATGCTC CTGTAGTCGCGGATGGACGAC TCGTTCTTGTTGCGGTAGCTCGGGGCGTCCGCGTCC GCGCTCTAGTGGGCGTGGTGG GACGTCT
5` P 3` OH
AM8BR2
5` P 3` OH
AM8F4
BstXI
HindIII
GCGACCAGGAGGAGATCGACTACGACGACACCATCAGCGTGGAAGCTTTAC
CGCTGGTCCTCCTCTAGCTGATGCTGCTGTGGTAGTCGCACCTTCGAAATG
AM8R1

FIG. 8

```
      EcoRI      NheI
   1  TAGAATTCGTAGGCTAGCATGCAGATCGAGCTGAGCACCTGCTTCTTCCTGTGCCTGCTGCGCTTCTGCTTC
                 1▶ MetGlnIleGluLeuSerThrCysPhePheLeuCysLeuLeuArgPheCysPhe
  73  AGCGCCACCCGCCGCTACTACCTGGGCGCCGTGGAGCTGAGCTGGGACTACATGCAGAGCGACCTGGGCGAG
  19▶ SerAlaThrArgArgTyrTyrLeuGlyAlaValGluLeuSerTrpAspTyrMetGlnSerAspLeuGlyGlu
 145  CTGCCCGTGGACGCCCGCTTCCCCCCCCGCGTGCCCAAGAGCTTCCCCTTCAACACCAGCGTGGTGTACAAG
  43▶ LeuProValAspAlaArgPheProProArgValProLysSerPheProPheAsnThrSerValValTyrLys
 217  AAGACCCTGTTCGTGGAGTTCACCGACCACCTGTTCAACATCGCCAAGCCCCGCCCCCCCTGGATGGGCCTG
  67▶ LysThrLeuPheValGluPheThrAspHisLeuPheAsnIleAlaLysProArgProProTrpMetGlyLeu
      ApaI                                                          MscI
 289  CTGGGCCCCACCATCCAGGCCGAGGTGTACGACACCGTGGTGATCACCCTGAAGAACATGGCCAGCCACCCC
  91▶ LeuGlyProThrIleGlnAlaGluValTyrAspThrValValIleThrLeuLysAsnMetAlaSerHisPro
 361  GTGAGCCTGCACGCCGTGGGCGTGAGCTACTGGAAGGCCAGCGAGGGCGCCGAGTACGACGACCAGACCAGC
 115▶ ValSerLeuHisAlaValGlyValSerTyrTrpLysAlaSerGluGlyAlaGluTyrAspAspGlnThrSer
 433  CAGCGCGAGAAGGAGGACGACAAGGTGTTCCCCGGCGGCAGCCACACCTACGTGTGGCAGGTGCTGAAGGAG
 139▶ GlnArgGluLysGluAspAspLysValPheProGlyGlySerHisThrTyrValTrpGlnValLeuLysGlu
          MscI                                                  PmlI
 505  AACGGCCCCATGGCCAGCGACCCCCTGTGCCTGACCTACAGCTACCTGAGCCACGTGGACCTGGTGAAGGAC
 163▶ AsnGlyProMetAlaSerAspProLeuCysLeuThrTyrSerTyrLeuSerHisValAspLeuValLysAsp
                                                              MscI
 577  CTGAACAGCGGCCTGATCGGCGCCCTGCTGGTGTGCCGCGAGGGCAGCCTGGCCAAGGAGAAGACCCAGACC
 187▶ LeuAsnSerGlyLeuIleGlyAlaLeuLeuValCysArgGluGlySerLeuAlaLysGluLysThrGlnThr
 649  CTGCACAAGTTCATCCTGCTGTTCGCCGTGTTCGACGAGGGCAAGAGCTGGCACAGCGAGACCAAGAACAGC
 211▶ LeuHisLysPheIleLeuLeuPheAlaValPheAspGluGlyLysSerTrpHisSerGluThrLysAsnSer
 721  CTGATGCAGGACCGCGACGCCGCCAGCGCCCGCGCCTGGCCCAAGATGCACACCGTGAACGGCTACGTGAAC
 235▶ LeuMetGlnAspArgAspAlaAlaSerAlaArgAlaTrpProLysMetHisThrValAsnGlyTyrValAsn
                                                          PmlI
 793  CGCAGCCTGCCCGGCCTGATCGGCTGCCACCGCAAGAGCGTGTACTGGCACGTGATCGGCATGGGCACCACC
 259▶ ArgSerLeuProGlyLeuIleGlyCysHisArgLysSerValTyrTrpHisValIleGlyMetGlyThrThr
 865  CCCGAGGTGCACAGCATCTTCCTGGAGGGCCACACCTTCCTGGTGCGCAACCACCGCCAGGCCAGCCTGGAG
 283▶ ProGluValHisSerIlePheLeuGluGlyHisThrPheLeuValArgAsnHisArgGlnAlaSerLeuGlu
 937  ATCAGCCCCATCACCTTCCTGACCGCCCAGACCCTGCTGATGGACCTGGGCCAGTTCCTGCTGTTCTGCCAC
 307▶ IleSerProIleThrPheLeuThrAlaGlnThrLeuLeuMetAspLeuGlyGlnPheLeuLeuPheCysHis
1009  ATCAGCAGCCACCAGCACGACGGCATGGAGGCCTACGTGAAGGTGGACAGCTGCCCCGAGGAGCCCCAGCTG
 331▶ IleSerSerHisGlnHisAspGlyMetGluAlaTyrValLysValAspSerCysProGluGluProGlnLeu
1081  CGCATGAAGAACAACGAGGAGGCCGAGGACTACGACGACGACCTGACCGACAGCGAGATGGACGTGGTGCGC
 355▶ ArgMetLysAsnAsnGluGluAlaGluAspTyrAspAspAspLeuThrAspSerGluMetAspValValArg
                                (BglII/BamHI)
1153  TTCGACGACGACAACAGCCCCAGCTTCATCCAGATCCGCAGCGTGGCCAAGAAGCACCCCAAGACCTGGGTG
 379▶ PheAspAspAspAsnSerProSerPheIleGlnIleArgSerValAlaLysLysHisProLysThrTrpVal
1225  CACTACATCGCCGCCGAGGAGGAGGACTGGGACTACGCCCCCCTGGTGCTGGCCCCCGACGACCGCAGCTAC
 403▶ HisTyrIleAlaAlaGluGluGluAspTrpAspTyrAlaProLeuValLeuAlaProAspAspArgSerTyr
                                    EagI
1297  AAGAGCCAGTACCTGAACAACGGCCCCCAGCGCATCGGCCGCAAGTACAAGAAGGTGCGCTTCATGGCCTAC
 427▶ LysSerGlnTyrLeuAsnAsnGlyProGlnArgIleGlyArgLysTyrLysLysValArgPheMetAlaTyr
                                                          ApaI
1369  ACCGACGAGACCTTCAAGACCCGCGAGGCCATCCAGCACGAGAGCGGCATCCTGGGCCCCCTGCTGTACGGC
 451▶ ThrAspGluThrPheLysThrArgGluAlaIleGlnHisGluSerGlyIleLeuGlyProLeuLeuTyrGly
```

FIG. 9A

```
1441 GAGGTGGGCGACACCCTGCTGATCATCTTCAAGAACCAGGCCAGCCGCCCCTACAACATCTACCCCCACGGC
 475▶GluValGlyAspThrLeuLeuIleIlePheLysAsnGlnAlaSerArgProTyrAsnIleTyrProHisGly
1513 ATCACCGACGTGCGCCCCCTGTACAGCCGCCGCCTGCCCAAGGGCGTGAAGCACCTGAAGGACTTCCCCATC
 499▶IleThrAspValArgProLeuTyrSerArgArgLeuProLysGlyValLysHisLeuLysAspPheProIle
                BglII
1585 CTGCCCGGCGAGATCTTCAAGTACAAGTGGACCGTGACCGTGGAGGACGGCCCCACCAAGAGCGACCCCCGC
 523▶LeuProGlyGluIlePheLysTyrLysTrpThrValThrValGluAspGlyProThrLysSerAspProArg
1657 TGCCTGACCCGCTACTACAGCAGCTTCGTGAACATGGAGCGCGACCTGGCCAGCGGCCTGATCGGCCCCCTG
 547▶CysLeuThrArgTyrTyrSerSerPheValAsnMetGluArgAspLeuAlaSerGlyLeuIleGlyProLeu
1729 CTGATCTGCTACAAGGAGAGCGTGGACCAGCGCGGCAACCAGATCATGAGCGACAAGCGCAACGTGATCCTG
 571▶LeuIleCysTyrLysGluSerValAspGlnArgGlyAsnGlnIleMetSerAspLysArgAsnValIleLeu
                      KpnI
1801 TTCAGCGTGTTCGACGAGAACCGCAGCTGGTACCTGACCGAGAACATCCAGCGCTTCCTGCCCAACCCCGCC
 595▶PheSerValPheAspGluAsnArgSerTrpTyrLeuThrGluAsnIleGlnArgPheLeuProAsnProAla
1873 GGCGTGCAGCTGGAGGACCCCGAGTTCCAGGCCAGCAACATCATGCACAGCATCAACGGCTACGTGTTCGAC
 619▶GlyValGlnLeuGluAspProGluPheGlnAlaSerAsnIleMetHisSerIleAsnGlyTyrValPheAsp
1945 AGCCTGCAGCTGAGCGTGTGCCTGCACGAGGTGGCCTACTGGTACATCCTGAGCATCGGCGCCCAGACCGAC
 643▶SerLeuGlnLeuSerValCysLeuHisGluValAlaTyrTrpTyrIleLeuSerIleGlyAlaGlnThrAsp
2017 TTCCTGAGCGTGTTCTTCAGCGGCTACACCTTCAAGCACAAGATGGTGTACGAGGACACCCTGACCCTGTTC
 667▶PheLeuSerValPhePheSerGlyTyrThrPheLysHisLysMetValTyrGluAspThrLeuThrLeuPhe
                                                BamHI
2089 CCCTTCAGCGGCGAGACCGTGTTCATGAGCATGGAGAACCCCGGCCTGTGGATCCTGGGCTGCCACAACAGC
 691▶ProPheSerGlyGluThrValPheMetSerMetGluAsnProGlyLeuTrpIleLeuGlyCysHisAsnSer
2161 GACTTCCGCAACCGCGGCATGACCGCCCTGCTGAAGGTGAGCAGCTGCGACAAGAACACCGGCGACTACTAC
 715▶AspPheArgAsnArgGlyMetThrAlaLeuLeuLysValSerSerCysAspLysAsnThrGlyAspTyrTyr
2233 GAGGACAGCTACGAGGACATCAGCGCCTACCTGCTGAGCAAGAACAACGCCATCGAGCCCCGCAGGCGCAGG
 739▶GluAspSerTyrGluAspIleSerAlaTyrLeuLeuSerLysAsnAsnAlaIleGluProArgArgArgArg
                                                BstXI
2305 CGCGAGATCACCCGCACCACCCTGCAGAGCGACCAGGAGGAGATCGACTACGACGACACCATCAGCGTGGAG
 763▶ArgGluIleThrArgThrThrLeuGlnSerAspGlnGluGluIleAspTyrAspAspThrIleSerValGlu
2377 ATGAAGAAGGAGGACTTCGACATCTACGACGAGGACGAGAACCAGAGCCCCCGCAGCTTCCAGAAGAAGACC
 787▶MetLysLysGluAspPheAspIleTyrAspGluAspGluAsnGlnSerProArgSerPheGlnLysLysThr
                                                PmlI
2449 CGCCACTACTTCATCGCCGCCGTGGAGCGCCTGTGGGACTACGGCATGAGCAGCAGCCCCCACGTGCTGCGC
 811▶ArgHisTyrPheIleAlaAlaValGluArgLeuTrpAspTyrGlyMetSerSerSerProHisValLeuArg
2521 AACCGCGCCCAGAGCGGCAGCGTGCCCCAGTTCAAGAAGGTGGTGTTCCAGGAGTTCACCGACGGCAGCTTC
 835▶AsnArgAlaGlnSerGlySerValProGlnPheLysLysValValPheGlnGluPheThrAspGlySerPhe
                                                ApaI
2593 ACCCAGCCCCTGTACCGCGGCGAGCTGAACGAGCACCTGGGCCTGCTGGGCCCCTACATCCGCGCCGAGGTG
 859▶ThrGlnProLeuTyrArgGlyGluLeuAsnGluHisLeuGlyLeuLeuGlyProTyrIleArgAlaGluVal
                BstEII
2665 GAGGACAACATCATGGTGACCTTCCGCAACCAGGCCAGCCGCCCCTACAGCTTCTACAGCAGCCTGATCAGC
 883▶GluAspAsnIleMetValThrPheArgAsnGlnAlaSerArgProTyrSerPheTyrSerSerLeuIleSer
2737 TACGAGGAGGACCAGCGCCAGGGCGCCGAGCCCCGCAAGAACTTCGTGAAGCCCAACGAGACCAAGACCTAC
 907▶TyrGluGluAspGlnArgGlnGlyAlaGluProArgLysAsnPheValLysProAsnGluThrLysThrTyr
2809 TTCTGGAAGGTGCAGCACCACATGGCCCCCACCAAGGACGAGTTCGACTGCAAGGCCTGGGCCTACTTCAGC
 931▶PheTrpLysValGlnHisHisMetAlaProThrLysAspGluPheAspCysLysAlaTrpAlaTyrPheSer
```

FIG. 9B

```
2881 GACGTGGACCTGGAGAAGGACGTGCACAGCGGCCTGATCGGCCCCCTGCTGGTGTGCCACACCAACACCCTG
 955▶AspValAspLeuGluLysAspValHisSerGlyLeuIleGlyProLeuLeuValCysHisThrAsnThrLeu
                    EagI          BstEII
2953 AACCCCGCCCACGGCCGCCAGGTGACCGTGCAGGAGTTCGCCCTGTTCTTCACCATCTTCGACGAGACCAAG
 979▶AsnProAlaHisGlyArgGlnValThrValGlnGluPheAlaLeuPhePheThrIlePheAspGluThrLys
3025 AGCTGGTACTTCACCGAGAACATGGAGCGCAACTGCCGCGCCCCCTGCAACATCCAGATGGAGGACCCCACC
1003▶SerTrpTyrPheThrGluAsnMetGluArgAsnCysArgAlaProCysAsnIleGlnMetGluAspProThr
3097 TTCAAGGAGAACTACCGCTTCCACGCCATCAACGGCTACATCATGGACACCCTGCCCGGCCTGGTGATGGCC
1027▶PheLysGluAsnTyrArgPheHisAlaIleAsnGlyTyrIleMetAspThrLeuProGlyLeuValMetAla
                          KpnI
3169 CAGGACCAGCGCATCCGCTGGTACCTGCTGAGCATGGGCAGCAACGAGAACATCCACAGCATCCACTTCAGC
1051▶GlnAspGlnArgIleArgTrpTyrLeuLeuSerMetGlySerAsnGluAsnIleHisSerIleHisPheSer
       PmlI
3241 GGCCACGTGTTCACCGTGCGCAAGAAGGAGGAGTACAAGATGGCCCTGTACAACCTGTACCCCGGCGTGTTC
1075▶GlyHisValPheThrValArgLysLysGluGluTyrLysMetAlaLeuTyrAsnLeuTyrProGlyValPhe
3313 GAGACCGTGGAGATGCTGCCCAGCAAGGCCGGCATCTGGCGCGTGGAGTGCCTGATCGGCGAGCACCTGCAC
1099▶GluThrValGluMetLeuProSerLysAlaGlyIleTrpArgValGluCysLeuIleGlyGluHisLeuHis
3385 GCCGGCATGAGCACCCTGTTCCTGGTGTACAGCAACAAGTGCCAGACCCCCCTGGGCATGGCCAGCGGCCAC
1123▶AlaGlyMetSerThrLeuPheLeuValTyrSerAsnLysCysGlnThrProLeuGlyMetAlaSerGlyHis
                                                  ApaI
3457 ATCCGCGACTTCCAGATCACCGCCAGCGGCCAGTACGGCCAGTGGGCCCCCAAGCTGGCCCGCCTGCACTAC
1147▶IleArgAspPheGlnIleThrAlaSerGlyGlnTyrGlyGlnTrpAlaProLysLeuAlaArgLeuHisTyr
3529 AGCGGCAGCATCAACGCCTGGAGCACCAAGGAGCCCTTCAGCTGGATCAAGGTGGACCTGCTGGCCCCCATG
1171▶SerGlySerIleAsnAlaTrpSerThrLysGluProPheSerTrpIleLysValAspLeuLeuAlaProMet
3601 ATCATCCACGGCATCAAGACCCAGGGCGCCCGCCAGAAGTTCAGCAGCCTGTACATCAGCCAGTTCATCATC
1195▶IleIleHisGlyIleLysThrGlnGlyAlaArgGlnLysPheSerSerLeuTyrIleSerGlnPheIleIle
3673 ATGTACAGCCTGGACGGCAAGAAGTGGCAGACCTACCGCGGCAACAGCACCGGCACCCTGATGGTGTTCTTC
1219▶MetTyrSerLeuAspGlyLysLysTrpGlnThrTyrArgGlyAsnSerThrGlyThrLeuMetValPhePhe
                                                    (SmaI/EcoRV)
3745 GGCAACGTGGACAGCAGCGGCATCAAGCACAACATCTTCAACCCCCCCATCATCGCCCGCTACATCCGCCTG
1243▶GlyAsnValAspSerSerGlyIleLysHisAsnIlePheAsnProProIleIleAlaArgTyrIleArgLeu
3817 CACCCCACCCACTACAGCATCCGCAGCACCCTGCGCATGGAGCTGATGGGCTGCGACCTGAACAGCTGCAGC
1267▶HisProThrHisTyrSerIleArgSerThrLeuArgMetGluLeuMetGlyCysAspLeuAsnSerCysSer
3889 ATGCCCCTGGGCATGGAGAGCAAGGCCATCAGCGACGCCCAGATCACCGCCAGCAGCTACTTCACCAACATG
1291▶MetProLeuGlyMetGluSerLysAlaIleSerAspAlaGlnIleThrAlaSerSerTyrPheThrAsnMet
3961 TTCGCCACCTGGAGCCCCAGCAAGGCCCGCCTGCACCTGCAGGGCCGCAGCAACGCCTGGCGCCCCCAGGTG
1315▶PheAlaThrTrpSerProSerLysAlaArgLeuHisLeuGlnGlyArgSerAsnAlaTrpArgProGlnVal
                                                    BstEII
4033 AACAACCCCAAGGAGTGGCTGCAGGTGGACTTCCAGAAGACCATGAAGGTGACCGGCGTGACCACCCAGGGC
1339▶AsnAsnProLysGluTrpLeuGlnValAspPheGlnLysThrMetLysValThrGlyValThrThrGlnGly
4105 GTGAAGAGCCTGCTGACCAGCATGTACGTGAAGGAGTTCCTGATCAGCAGCAGCCAGGACGGCCACCAGTGG
1363▶ValLysSerLeuLeuThrSerMetTyrValLysGluPheLeuIleSerSerSerGlnAspGlyHisGlnTrp
4177 ACCCTGTTCTTCCAGAACGGCAAGGTGAAGGTGTTCCAGGGCAACCAGGACAGCTTCACCCCCGTGGTGAAC
1387▶ThrLeuPhePheGlnAsnGlyLysValLysValPheGlnGlyAsnGlnAspSerPheThrProValValAsn
4249 AGCCTGGACCCCCCCCCTGCTGACCCGCTACCTGCGCATCCACCCCCAGAGCTGGGTGCACCAGATCGCCCTG
1411▶SerLeuAspProProLeuLeuThrArgTyrLeuArgIleHisProGlnSerTrpValHisGlnIleAlaLeu
                                                    SmaI          HindIII
4321 CGCATGGAGGTGCTGGGCTGCGAGGCCCAGGACCTGTACTAGCTGCCCGGGCTACAAGCTTTAC
1435▶ArgMetGluValLeuGlyCysGluAlaGlnAspLeuTyr•••
```

FIG. 9C

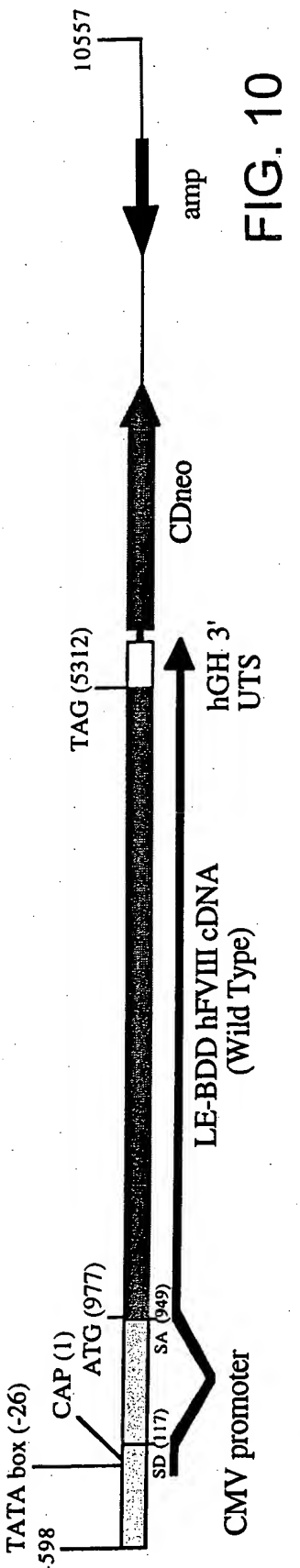


FIG. 10

FIG. 11

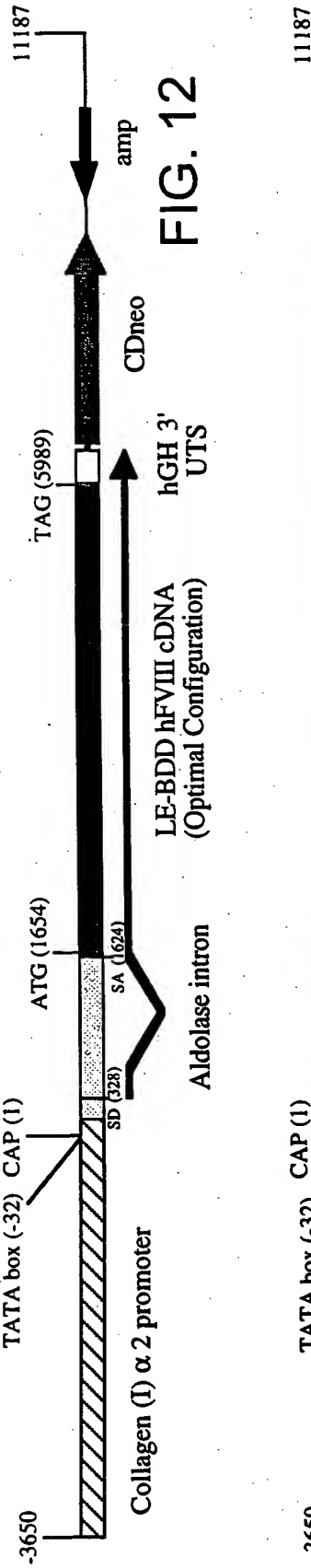


FIG. 12

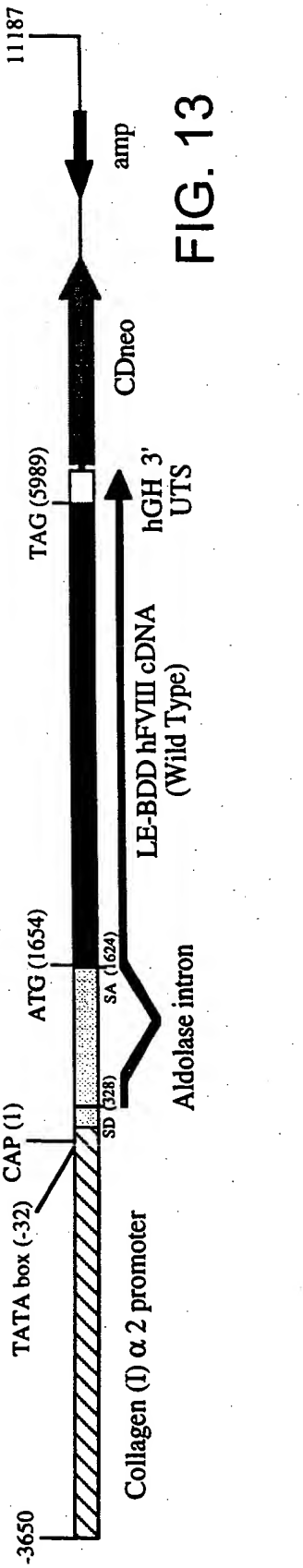


FIG. 13

Codon Frequency in Highly Expressed Human Genes

		% occurrence			% occurrence			% occurrence
Glu			Cys			Gln		
GA	A	25	TG	C	68	CA	A	12
	G	75		T	32		G	88
Arg			Ala			Gly		
CG	C	37	GC	C	53	GG	C	50
	T	7		T	17		T	12
	A	6		A	13		A	14
	G	21		G	17		G	24
AG	A	10						
	G	18						
Leu			Ser			Pro		
CT	C	26	TC	C	28	CC	C	48
	T	5		T	13		T	19
	A	3		A	5		A	16
	G	58		G	9		G	17
TT	A	2	AG	C	34			
	G	6		T	10			

FIG. 14A

Codon Frequency in Highly Expressed Human Genes

		% occurrence			% occurrence			% occurrence
Ilc			Thr			Val		
AT	C	77	AC	C	57	GT	C	25
	T	18		T	14		T	7
	A	5		A	14		A	5
				G	15		G	64
Tyr			Phe			Lys		
TA	C	74	TT	C	80	AA	A	18
	T	26		T	20		G	82
Asn			His					
AA	C	78	CA	C	79			
	T	25		T	21			

FIG. 14B